Iridium- and Ruthenium-Catalyzed N-alkylation of Amines with Alcohols and Amines

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PhD thesis

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February 2012

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Technical University of Denmark

Preface

This thesis describes the work carried out at the Department of Chemistry at the Technical University of Denmark from January 2007 to February 2012 supervised by Professor Robert Madsen. In that period a 6 month external stay at The Scripps Research Institute, San Diego, California, USA, from September 2008 till the end of February 2009 was conducted under supervision by Professor Phillip E. Dawson.

Firstly, I would like to thank Robert Madsen for always having time to discuss chemistry or personal issues during the five years I spent in his group. I am very grateful for his patience with me, as my studies have been interrupted twice due to maternity leaves. He has been a great mentor and has in particular taught me how to maintain focus and not to “run off track”. I am also very grateful to Philip E. Dawson for hosting my external stay and for introducing me to the field of peptide/protein synthesis. It opened my eyes to an area I was otherwise very skeptical at. Despite the many technical troubles I had with the machinery, the outcome of the project at a personal level was very fruitful. The whole Dawson group is thanked for teaching me the basics of peptide synthesis. Additionally, I thank Associate Professor Evan Powers from the Kelly Lab for conducting the computational fitting of my data.

I have experience both good and bad years as a PhD student. The bad years were when chemistry did not act as I wanted and the number of fellow students was low. Obviously, the good years were when chemistry was successful, but also when the building was full of students and the frustrations over lack of good results drowned in great company. Luckily, the last year was a blast and in particular the “jyder” are major contributors. I have had the pleasure of sharing not only one lab but three different labs with many people. Thanks to Lars Linderoth, Ilya Markarov, Lasse B. Olsen, Caroline Møller, Rasmus Aniol and Esben Olsen for good company in the labs. Thanks to the rest of building 201 and in particular to former technicians Ulla Maxmiling for assistance with experiments during my first pregnancy and to Janne Borg Rasmussen for assistant with everything from finding a chemical to explanation of waste handling and many joyful chats. Signe Teuber Seger (previously Henriksen) is thanked for “girl support” when I started my PhD studies and the majority of the students were of the male gender. The relationship has continued after her departure from DTU and she is now a good friend and she is also thanked for proof reading this thesis.

Finally, I express my deepest gratitude to my dear husband André for support and understanding during the years. My parents are greatly appreciated for babysitting my two adorable and very energetic boys for what seems like an uncountable number of hours. My dad is also thanked for allowing me to set up camp during the months of thesis writing and hereby interrupting his otherwise peaceful retirement.

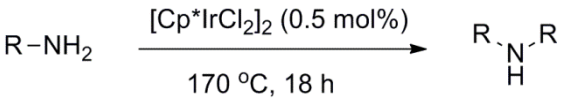
Funding of my PhD stipend from the Danish National Research Foundation is greatly appreciated as well as the following foundations for financial support of my external stay: Augustinus Fonden, Fabrikant P.A. Fiskers Fond, Idella Fonden, Ingeniør Alexandre Haynman og hustru Nina Haynmans Fond, Knud Højgårds Fond, Kemisk Forenings Rejsefond, Oticon Fonden, Otto Mønsteds Fond and Rudolph Als Fondet.

Linda Luise Reeh Lorentz-Petersen
Kgs. Lyngby, February 2012

Abstract

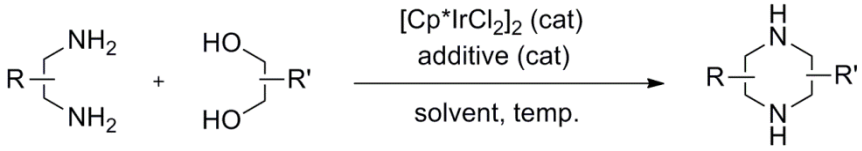
Many biologically active molecules contain one or more nitrogen atoms. Consequently, C-N bond formation is a crucial area in the development of pharmaceuticals. The main part of this thesis is devoted to environmentally benign syntheses of different nitrogen scaffolds. Iridium and ruthenium catalysts have been employed for the N-alkylation of amines with either alcohols or amines.

Synthesis of secondary amines



Self-condensation of primary amines afforded secondary amines in good to high yields. The reaction is catalyzed by the commercially available $[Cp^*IrCl_2]_2$ complex. The procedure is environmentally benign as it is performed in the absence of both solvent and additives and the only by-product is ammonia. Additionally, the work-up procedure is a simple distillation of the product directly from the reaction mixture.

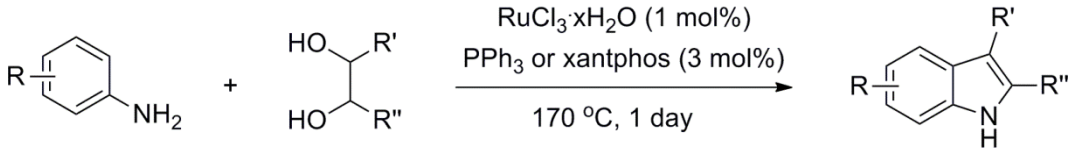
Synthesis of piperazines



In the Madsen group it has previously been demonstrated that condensation of diamines and diols catalyzed by $[Cp^*IrCl_2]_2$ furnishes the piperazine skeleton. The only by-product of the reaction is water. The substrate scope was extended and the limitations of the reaction were studied. It was established that the Thorpe-Ingold effect plays a central role in the reaction, as ethyleneglycol and 1,2-ethylenediamine failed to produce piperazine. Introduction of a C-substituent on one or both of the starting materials gave C-substituted piperazines in high yields. Synthesis of N-benzylpiperazine from ethyleneglycol and N-benzylethylenediamine was also successful. Self-condensation of ethanolamine was unsuccessful due to polymerization of the starting material. *o*-Phenylenediamine was a difficult substrate as it furnished an equimolar mixture of 1,2,3,4-tetrahydroquinoxaline and 2-benzimidazolemethanol in the reaction with ethylene glycol. Ammonium tetrafluoroborate as the nitrogen source in reaction with 1,2-cyclohexanediol afforded the morpholine derivative. Finally, attempts to switch to ruthenium catalysis were unsuccessful since neither a $RuCl_3$-PPh_3 complex nor a $RuCl_3$-xantphos complex was able to catalyze the reaction between 1,2-diaminocyclohexane

and ethylene glycol. Mechanistic experiments of the iridium catalyzed reactions revealed that the Voigt isomerization of the α-imino alcohol intermediate to the corresponding α-imino ketone plays a significant role.

Synthesis of indoles



Anilines and vicinal diols were reacted in the presence of a ruthenium complex ($RuCl_3$ with PPh_3 or xantphos) to give indoles in good yields. In this case water and dihydrogen are the only by-products. When unsymmetrical diols were employed the corresponding indole with the largest substituent in the 2-position was favoured. It is believed to proceed through a Bischler-like reaction pathway. Mechanistic experiments were conducted and emphasized the importance of the Voigt reaction in the formation of the product.

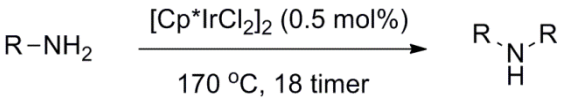
Protein folding

During an external stay at The Scripps Research Institute, San Diego, California, USA, folding of the well-known protein CI2 was studied. Several mutants were synthetically prepared via folding assisted ligation. One segment was synthesised as the C-terminal thioester by Boc-SPPS and the other segment as a C-terminal acid by Fmoc-SPPS. The sites of mutation were all in the α-helical region of the protein and the mutation choices were alanine and Aib, which both posses a high α-helical propensity. Hereby, it was believed that more stable proteins would be obtained. Folding of the mutants was studied in terms of thermodynamics and kinetics by guanidine hydrochloride denaturation monitored by fluorescence. The results were unfortunately unreliable due to errors in the spectrofluorometer.

Resumé

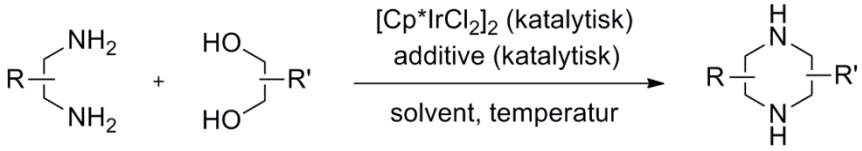
Mange biologisk aktive molekyler indeholder et eller flere nitrogen atomer. Derfor er dannelse af C-N bindinger et yderst vigtigt felt indenfor udvikling af medicin. Hoveddelen af denne afhandling omhandler miljøvenlige synteser af forskellige nitrogenindeholdende byggeblokke. Iridium og ruthenium katalysatorer er blevet anvendt til N-alkylering af aminer med enten alkoholer eller aminer.

Syntese af sekundære aminer



Selvkondensation af primære aminer gav sekundære aminer in gode eller høje udbytter. Reaktionen er katalyseret af det kommercielt tilgængelige $[Cp^*IrCl_2]_2$ kompleks. Proceduren er miljøvenlig, da den udføres uden tilstedeværelse af solvent eller additiver, og det eneste biprodukt er ammoniak. Ydermere er oparbejdningsmetoden en simpel destillation af produktet direkte fra reaktionsblandingen.

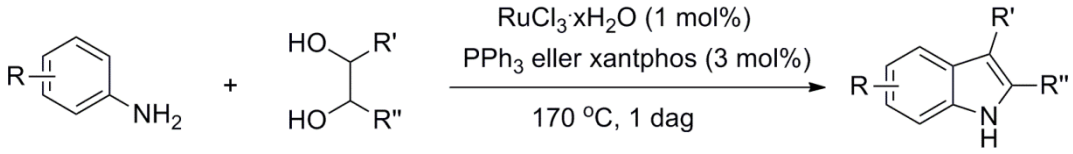
Syntese af piperaziner



I Madsen-gruppen er det tidligere blevet påvist, at kondensation af diaminer og dioler katalyseret af $[Cp^*IrCl_2]_2$ giver piperaziner, og det eneste biprodukt er vand. Substrattolerancen er blevet udvidet og begrænsningerne af reaktionen er blevet undersøgt. Det blev fastslået, at Thorpe-Ingold effekten spiller en central rolle i reaktionen, da ethylenglykol og 1,2-ethylendiamin ikke som forventet gav piperazine. Indførelse af en C-substituent på det ene eller begge af udgangsstofferne gav C-substituerede piperaziner i høje udbytter. Syntese af N-benzylpiperazin ud fra ethylenglykol og N-benzylethylendiamin var ligeledes succesfyldt. Selvkondensation af ethanolamin til piperazin fejlede pga. polymerisering af udgangsstoffet. *o*-Phenylethylendiamin var et vanskeligt udgangsstof, da det gav en ækvimolær blanding af 1,2,3,4-tetrahydroquinoxalin og 2-benzimidazolmethanol i reaktion med ethylenglykol. Ammonium tetrafluoroborat som nitrogenkilde gav i reaktion med 1,2-cyklohexandiol morfolinderivatet. Endeligt blev det forsøgt at anvende ruthenium katalyse, men det var uden succes, da hverken et $RuCl_3$-PPh_3 kompleks eller et $RuCl_3$-xantphos kompleks var i stand til at katalysere reaktionen mellem 1,2-diamincyclohexan og ethylenglykol.

Mekanistiske eksperimenter af de iridiumkatalyserede reaktioner afslørede at Voigt isomeriseringen af α-iminoalkohol-intermediatet til den tilsvarende α-iminoketon spiller en væsentlig rolle.

Syntese af indoler



Reaktion mellem aniliner og vicinale dioler under tilstedeværelse af et rutheniumkompleks ($RuCl_3$ med PPh_3 eller xantphos) gav indoler i gode udbytter. I dette tilfælde er vand og dihydrogen de eneste biprodukter. Når asymmetriske dioler blev anvendt, var den tilsvarende indol med den største substituent i 2-positionen favoriseret. Det menes at forløbe via et Bischler-ligende reaktionsforløb. Mekanistiske eksperimenter blev udført og understregede vigtigheden af Voigt reaktionen under dannelsen af produktet.

Proteinfoldning

Under et eksternt ophold ved The Scripps Research Institute, San Diego, Californien, USA blev foldning af det velkendte protein CI2 studeret. Adskellige mutanter blev syntetisk fremstillet via foldningsassisteret ligering. Det ene segment blev syntetisert som en C-terminal thioester via Boc-fastfase peptid syntese og det andet som en C-terminal syre via Fmoc-fastfase peptid syntese. Alle de muterede enheder er at finde i proteinets α-helix, og valget af mutant var alanin eller Aib, som begge besider en høj tilbøjelighed til at danne α-helixer. Hermed var det antaget, at mere stabile proteiner ville kunne opnås. Foldning af mutanterne med henblik på termodynamik og kinetik blev undersøgt gennem guanidinhydroklorid denaturering monitoreret af fluorescens. Desværre var resultaterne uanvendelige pga. fejl i spektrofluorometeret.

List of abbreviations

A	Alanine
Ac	Acetyl
acac	Acetylacetonate
Acpc	1-Aminocyclopropanecarboxylic acid
ADHD	Attention deficit hyperactivity disorder
Aib	α-Aminoisobutyric acid
aq.	Aqueous
Ar	Aromatic
ax	Axial
b	Broad (NMR)
BINAP	2,2'-Bis(diphenylphosphino)-1,1'- binaphtyl
Bn	Benzyl
Boc	*tert*-Butoxycarbonyl
Bp	Boiling point
Bu	Butyl
CI2	Chymotrypsin inhibitor
Clz	2-Chlorobenzyloxycarbonyl
cod	1,5-Cyclooctadiene
cot	1,3,5,7-Cyclooctatetraene
Cp	Cyclopentadienyl
Cp*	pentamethylcyclopentadienyl
Cy	Cyclohexyl
cymene	4-Isopropyltoluene
cyp	Cyclopentyl
d	Doublet (NMR)
D	Aspertic acid
dba	Dibenzylideneacetone
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DIC	N,N'-diisopropylcarbodiimide
DIEA	Diisopropylethylamine
DMAP	4-(Dimethylamino)pyridine
DME	Dimethoxyethane
DMF	*N,N'*-Dimethylformamide
DMSO	Dimethyl sulfoxide

DPEphos	1,1’-Bis(diphenylphospino)ether
dppf	1,1’-Bis(dihenylphosphino)ferrocene
E	Electrophile or glutamic acid
eq	Equivalents or equiratorial
ESI	Electrospray Ionization
F	Phenylalanine
Fmoc	9-Fluorenylmethoxycarbonyl
frac	Fraction
G	Glycine
GC	Gas chromatography
Gdn, Gdm	Guanidine
Gly	Glycine
h	hours
HATU	O-(7-Azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluoro-phosphate
HCTU	1-[Bis(dimethylamino)-methylene]-6-chloro-1*H*-benzotriazolium hexafluoro-phosphate 3-oxide
HIV	Human Immunodeficiency Virus
HMPA	Hexamethylphosphoramide
HOBt	1-Hydroxybenzotriazole
HPLC	High performance liquid chromatography
HRMS	High resolution mass spectroscopy
i	Iso
I	Isoleucine
Iphos	2,2’-Bis[di(3,5-trifluoromethylphenyl)phosphinomethyl]-1,1’-binaphthyl
K	Lysine
kcal	kilo calories
L	Leucine
Ln	Ligand
m	Multiplet (NMR)
M	Molar or methionine
Me	Methyl
Mp	Melting point
Ms	Mass spectroscopy
MsOH	Methanesulphonic acid
MW	Micro waves

N	Aspargine
NHC	Carbene ligand
NMM	N-Methylmorpholine
NMR	Nuclear magnetic resonance
Nos	2-Nitrophenysulfonyl
o	Ortho
Ox	Oxidation
p	Para
P	Proline
Pbf	2,2,4,6,7- Pentamethyldihydrobenzofurane
Ph	Phenyl
ppm	Parts per million
Py	Pyridine
q	Quartet (NMR)
Q	Glutamine
R	Arginine
r.t.	Room temperature
Red	Reduction
RP	Reversed phase
S	Serine
SPPS	Solid phase peptide synthesis
t	Triplet (NMR)
t, tert	Tertiary
T	Threonine
TEA	Triethylamine
TEBA	Triethylbenzylammonium chloride
Temp	Temperature
TES	Triethylsilyl
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TIS	Triisopropylsilane
TLC	Thin layer chromatography
TMEDA	Tetramethylethylenediamine
TMS	Tetramethylsilane or trimethylsilyl
tol	Tolyl
Trt	Trityl

TSA	Toluenesulfonic acid
UV	Ultraviolet
V	Valine
W	Tryptophan
wt	Wild type
Xan	S-xanthenyl
Xantphos	4,5-Bis(diphenylphosphino)-9-9-dimethylxanthene
Xphos	2-Dicyclohexylphosphino-2’,4’,6’-triisopropylbiphenyl
Y	Tyrosine
PPA	Polyphosphoric acid

Table of contents

1 Introduction

1.1 Green chemistry

Recently, the term 'green' has been used to great extend. It often refers to the areas of fuel (cars, planes and ships) and electrical power (coal, wind and nuclear). Most people do not consider the amounts of products that they are surrounded by which are directly linked to chemical synthesis. Chemicals are found in a large variety of products such as food, clothes, furniture, toys, drugs, shampoos, toothpastes, etc. One can choose the type of transportation and hereby directly affect the environment. However, it is more difficult to have a direct influence on the choice of products in the household despite the growing range of organic products. For instance, shampoos and wash detergents are made by a several manufacturers. Some have focused on the cheapest production process, which often to some extend ignore the effect of the production on the environment. Others have great focus on the environment both in terms of the production as well as when the product is exposed in the nature. Hereby, the consumer can directly choose to support environmentally friendly products and productions or not. Unfortunately, in regards to pharmaceuticals there is not such a choice. Often there is only one manufacturer, unless the patent has expired and a generic drug is available, but there is never a choice between a less and a more environmentally friendly drug. The responsibility lies therefore completely with the pharmaceutical companies as well as manufacturers of bulk and fine chemicals. Fortunately, they *do* have focus on green chemistry[1-4] as productions of these three product types are associated with large amounts of waste. A way to describe this waste problem is the E factor (= environmental factor), which was defined by Roger Sheldon in 1992 as kilogram waste per kilogram product.[5] In table 1 some of these E-factors are listed.

Table 1. E-factors in chemical industries.[6,7]

Product	Production per year (ton)	E factor
Oil refining	10^6-10^8	<0.1
Bulk chemicals	10^4-10^6	<1-5
Fine chemicals	10^2-10^4	5 -<50
Pharmaceuticals	10-10^3	25-<100

The numbers strongly indicate the need for improvements. Not only are the E factors high (in particular for pharmaceuticals) but the production sizes are also large and in turn this

makes the total amount of waste enormous. It should be noted that the E factor does not take into account the nature of the waste. Not all types of waste are a potential problem and in some cases the waste can be sold as a chemical to other industries or reused in the given process.

Waste is obviously a major concern in a chemical production, nevertheless other aspects of green chemistry should be taken into account. In the pharmaceutical industry, that has the highest E factor, the synthetic pathway to the final product should be carefully chosen.[8] In 1998 Anastas and Warner described "the twelve principles of green chemistry":[9]

1. It is better to prevent waste than to treat or clean up waste after it is formed.
2. Synthetic methods should be designed to maximize the incorporation of all materials used in the process into the final product.
3. Wherever practicable, synthetic methodologies should be designed to use and generate substances that possess little or no toxicity to human health and the environment.
4. Chemical products should be designed to preserve efficacy of function while reducing toxicity.
5. The use of auxiliary substances (e.g. solvents, separation agents, etc.) should be made unnecessary whenever possible and, innocuous when used.
6. Energy requirements should be recognized for their environmental and economic impacts and should be minimized. Synthetic methods should be conducted at ambient temperature and pressure.
7. A raw material feedstock should be renewable rather than depleting whenever technically and economically practical.
8. Unnecessary derivatization (blocking group, protection/deprotection, temporary modification of physical/chemical processes) should be avoided whenever possible.
9. Catalytic reagents (as selective as possible) are superior to stoichiometric reagents.
10. Chemical products should be designed so that at the end of their function they do not persist in the environment and break down into innocuous degradation products.
11. Analytic methodologies need to be further developed to allow for real-time in-process monitoring and control prior to the formation of hazardous substances.

12. Substances and the form of a substance used in a chemical process should be chosen so as to minimize the potential for chemical accidents, including releases, explosions, and fires.

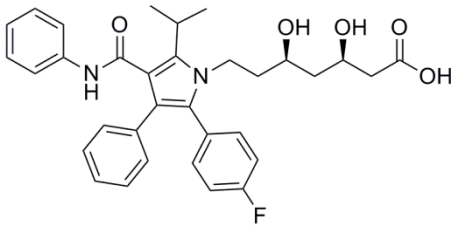
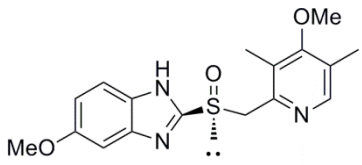
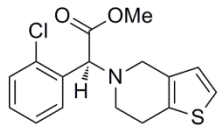
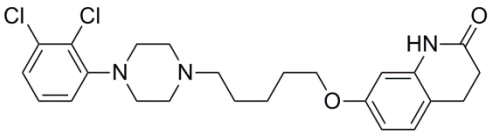
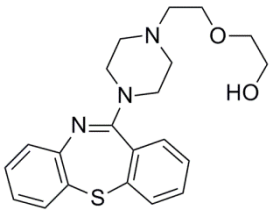
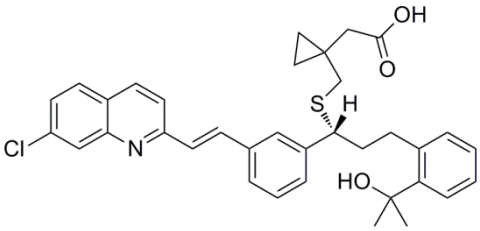
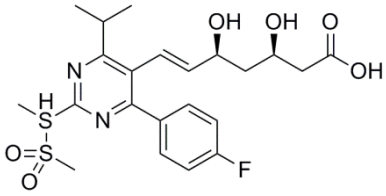
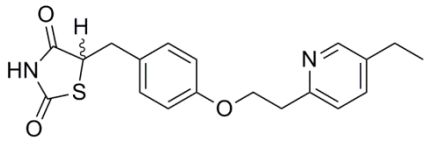
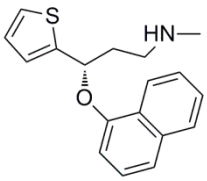
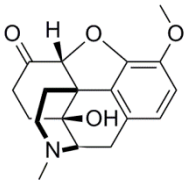
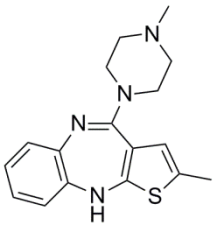
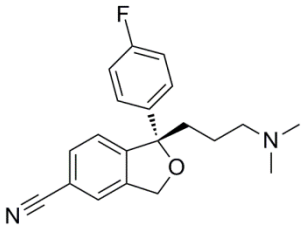
In an industrial setting each of the twelve principles should be revised carefully. However, in this thesis focus will predominantly lie on the following:

- Minimizing the number of chemical transformations
- Ensure good atom economy[10]
- Aiming for a low E-factor
- Minimizing hazardous chemicals/solvents
- Employment of catalytic reactions
- Short reaction time

1.2 Amines in the pharmaceutical industry

Organic compounds containing nitrogen are very important in the pharmaceutical industry. A total of 12 out of the 20 top selling drugs (in the US) in 2010 contained an "amine" function (linear, cyclic or heteroaromatic – not including biologically manufactured pharmaceuticals).[11] These are shown in table 2. In particular the cyclic amines are of great importance as they represent 50% of the top 20 selling drugs in the US.

Table 2. Amine containing pharmaceuticals.

#[a]	Chemical structure	#[a]	Chemical structure
1	 Lipitor by Pfizer (for high cholesterol)	2	 Nexium by AstraZeneca (e.g. for peptic ulcer disease)
3	 Plavix by Bristol-Myers Squibb/Sanofi-Aventis (for inhibition of blood cloths)	5	 Abilify by Otsuka (e.g. for schizophrenia and depression)
6	 Seroquel by AstraZeneca (for schizophrenia and bipolar disorder)	7	 Singulair by Merck (e.g. for asthma)
8	 Crestor by AstraZeneca (for high cholesterol)	9	 Actos by Takeda (for diabetes)
13	 Cymbalta by Lilly (e.g. for depression)	15	 Oxycontin by Purdue (for pain)
17	 Zyprexa by Lilly (for schizophrenia and bipolar disorder)	19	 Lexapro by Forest (for depression)

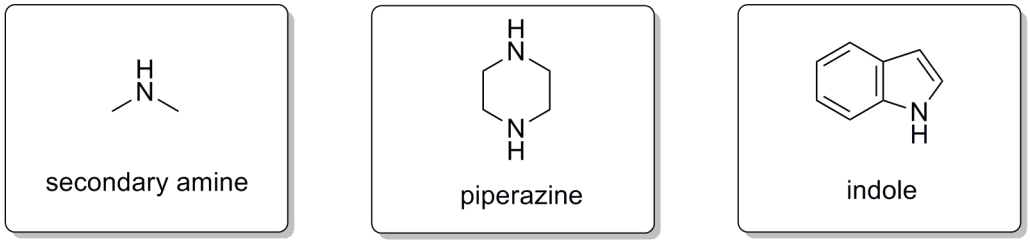
a: The numbers indicate the position on the list of the 20 top selling drugs

1.3 Privileged structures

The term 'privileged structure' was introduced by Evans in 1988.[12] A privileged structure is a molecular scaffold that is highly represented in biologically active compounds.[12-14] The substructure must be an essential core of the molecule for it to be considered a privileged structure. E.g. a simple amine is not considered a privileged structure despite its wide occurrence in biologically active compounds.[14] During the years many privileged structures have been identified such as benzodiazepines, purines, benzyl- and spiropiperidines, indoles, benzimidazoles, benzofuranes and -pyranes, biphenyltetrazoles, piperazines and cyclic peptides.[12-17]

This thesis will describe synthesis of the simple symmetric secondary amine as well as the privileged structures indoles and piperazines (figure 1).

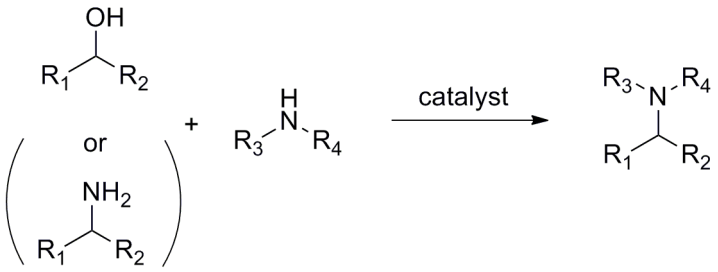
Figure 1. The chosen scaffolds**.**



Primarily the work done in the area of carbon-nitrogen bond formation by metal catalysis will be described. Focus is on reaction of amines in either self-condensation reactions or in reaction with alcohols to produce the compounds illustrated in figure 1.

1.4 Metal-catalyzed hydrogen transfer reaction of amines and alcohols

The reactions examined in this thesis are all metal-catalyzed hydrogen transfer reactions; more specifically they are N-alkylation of amines with alcohols or amines by the "borrowing hydrogen methodology". A generalized example is illustrated in scheme 1.



Scheme 1. N-alkylation of amines with alcohols (or amines).

This type of transformation has been studied for several decades with different catalysts. Attention has predominantly been given to the reaction between amine and alcohol. The metals that have shown the most promising results are ruthenium and iridium. Ruthenium was one of the front runners with the first publications in 1981[18,19] and in 2003 the first iridium catalyzed reaction was published[20]. Ruthenium catalysts are generally cheaper compared to iridium catalysts. Initially, the reaction conditions for the ruthenium catalyzed reactions were rather harsh and often the substrate scope was limited and therefore iridium catalysis was investigated.[21]

Besides the obvious formation of secondary and tertiary amines the method is also used for synthesis of heterocycles such as pyrrolidines, piperidines, azepanes, piperazines, indoles, quinolines, quinoxalines and benzimidazoles.[21-24] Much attention is given to the discovery of a catalytic system and optimum reaction conditions that furnish products with a wide range of substituents. Additionally, focus is on environmentally benign methods. So far there have been several reports on methods with a limited substrate scope. For instance, in syntheses of non-cyclic amines not all catalytic systems (or reaction conditions) allow all types of substrates. Some methods are not compatible with aryl amines, while others do not work with secondary alcohols.

In table 3 to 6 all known N-alkylations of amines with alcohols are listed, according to the best of our knowledge. Table 3 describes the formation of linear secondary and tertiary amines, table 4 the formation of N-heterocycles, table 5 the formations of aromatic N-heterocycles and finally all transformations involving ammonia or ammonium salts are listed in table 6.

Table 3. Formation of linear secondary and tertiary amines.

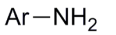
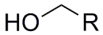
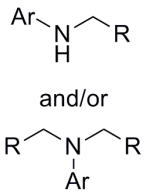
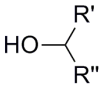
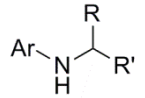
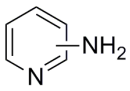
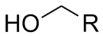
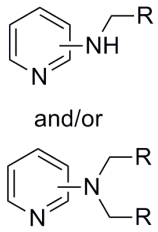
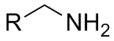
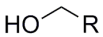
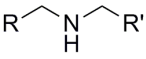
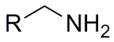
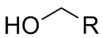
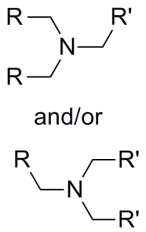
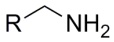
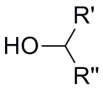
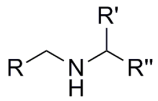
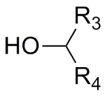
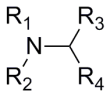
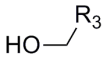
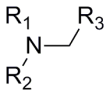
#	Amine	Alcohol	Product	Ru-catalyst	Ir-catalyst
1				$RuCl_2(PPh_3)_3$[18,25] $RuCl_3/P(OBu)_3$[26] $[RuCl(PPh_3)_2(CH_3CN)_3]^+[BPh_4]^-$[27] $[Ru(p\text{-cymene})Cl_2]_2$[28,29]	$[Cp^*IrCl_2]_2$[20,30] $[IrCl_2Cp^*(NHC)]$[31] [Ir]/P,N-ligand[32-35] $[Cp^*Ir(NH_3)_3][I]_2$[36]
2				$RuCl_2(PPh_3)_3$[18]	$[Cp^*IrCl_2]_2$[20] $[Cp^*Ir(NH_3)_3][I]_2$[36]
3				Ru(cod)(cot)[37] $RuCl_2(PPh_3)_3$[37]	[Ir]/P,N-ligand[32-35]
4				$RuH_2(PPh_3)_4$[38] $[Ru(p\text{-cymene})Cl_2]_2$[28,29,39]	$[Cp^*IrCl_2]_2$[20,30] $[IrCl_2Cp^*(NHC)]$[31] $Cp^*Ir(NH_3)_3][I]_2$[36]
5				$RuCl_2(PPh_3)_3$[40]	Ir-Pincer[41] $[IrCl_2Cp^*(NHC)]$[31]
6				$RuH_2(PPh_3)_4$[38] $Ru_3(CO)_{12}$/ligand[42]	$[Cp^*IrCl_2]_2$[20] $[IrCl_2Cp^*(NHC)]$[31]
7				$Ru_3(CO)_{12}$/ligand[43,44] $[Ru(p\text{-cymene})Cl_2]_2$[28,45]	$[Cp^*IrCl_2]_2$[30] $[IrCl_2Cp^*(NHC)]$[31]
8				$RuCl_2(PPh_3)_3$[46] $RuCl_3$/dppf[47] $[Ru(p\text{-cymene})Cl_2]_2$[28,29,45] $Ru_3(CO)_{12}$/ligand[44] Shvo[48]	$[Cp^*IrCl_2]_2$[30,49] $[Cp^*Ir(NH_3)_3][I]_2$[36] Ir-Pincer[41]

Table 4. Formation of N-heterocycles.

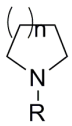
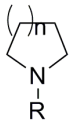
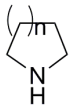
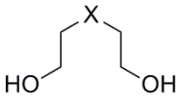
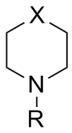
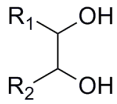
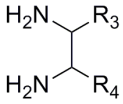
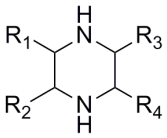
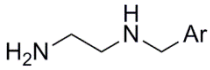
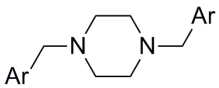
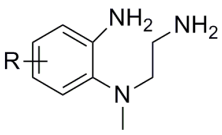
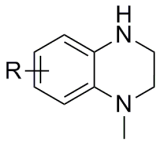
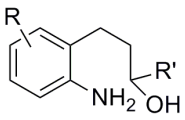
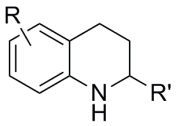
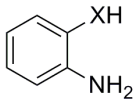
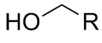
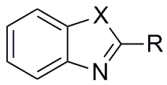
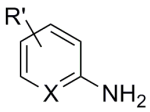
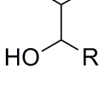
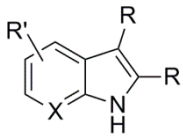
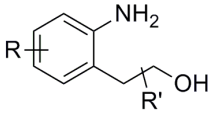
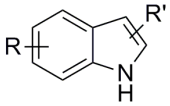
#	Amine	Alcohol	Product	Ru-catalyst	Ir-catalyst
1		$R-NH_2$		$RuCl_2(PPh_3)_3$[50] $RuH_2(PPh_3)_4$[38] [Ru(*p*-cymene)Cl_2]$_2$[28,29]	$[Cp^*IrCl_2]_2$[49,51-54] $[Cp^*Ir(NH_3)_3][I]_2$[36]
2		R–OH		$RuH_2(PPh_3)_4$[38]	-
3		-		$RuH_2(PPh_3)_4$[38]	-
4	 X = CH_2, NR', O	$R-NH_2$		$RuCl_2(PPh_3)_3$[55] $RuCl_3/PBu_3$[55] Ru-complex[56]	-
5				$Ru_3(CO)_{12}/PBu_3$[57]	-
6		-		-	$[Cp^*IrCl_2]_2$[58]
7		-		-	$[Cp^*IrCl_2]_2$[59]
8		-		-	$[Cp^*IrCl_2]_2$[60]

Table 5. Formation of aromatic N-heterocycles.

#	Amine	Alcohol	Product	Ru-catalyst	Ir-catalyst
1				X=NH,O: $RuCl_2(PPh_3)_3$[61] X=NH:$Ru(PPh_3)_3(CO)H_2$/ligand[62] X=O: Shvo[63]	
2	 X = C, N			X=C: $RuCl_2(PPh_3)_3$[64] X=N:$RuCl_2(PPh_3)_3$[65, 66]	-
3				$RuCl_2(PPh_3)_3$[67, 68]	$[Cp^*IrCl_2]_2$[60]

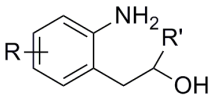
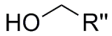
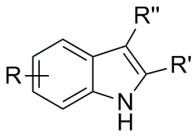
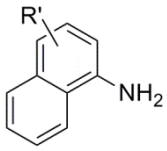
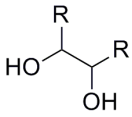
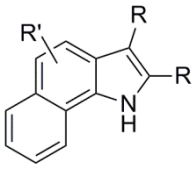
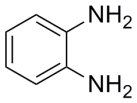
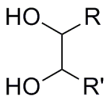
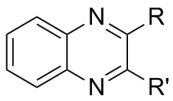
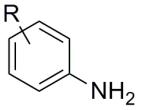
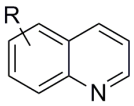
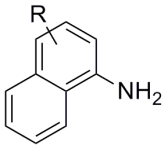
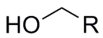
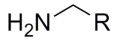
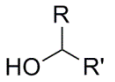
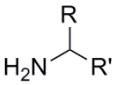
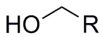
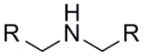
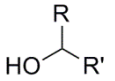
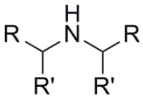
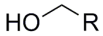
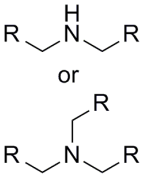
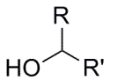
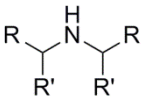
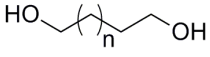
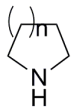
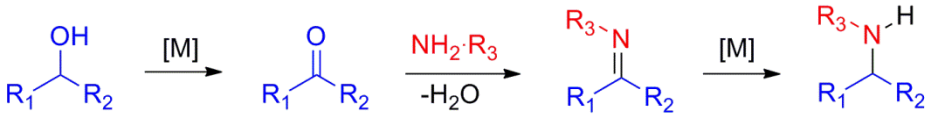
4				-	$[Cp^*IrCl_2]_2$[69]
5				-	$IrCl_3$/BINAP[70]
6				$RuCl_2(PPh_3)_3$[71]	-
7				$RuCl_3/PBu_3$[64]	-
8				-	$IrCl_3$/BINAP[70]

Table 6. Transformations with ammonia and ammonium salts.

	Amine	Alcohol	Product	Ru-catalyst	Ir-catalyst
1	NH_3			Ru/ligand[72]	-
2	NH_3			$Ru_3(CO)_{12}$/ligand[73] $Ru(CO)ClH(PPh_3)_3$/ligand[74]	-
3	NH_3			-	$[Cp^*Ir(NH_3)_3][I]_2$[75]
4	NH_3			-	$[Cp^*Ir(NH_3)_3][I]_2$[75]
5	NH_4X			-	$[Cp^*IrCl_2]_2$[76, 77]
6	NH_4X			-	$[Cp^*IrCl_2]_2$[76, 77]
7	NH_4X			-	$[Cp^*IrCl_2]_2$[76]

1.4.1 Proposed reaction mechanism

Ruthenium and iridium catalyzed N-alkylation of amines with alcohols involve the same key steps (scheme 2). Firstly, the alcohol is oxidized to the carbonyl, then a reaction with the amine to give the imine upon removal of water, and finally reduction of the imine to the secondary amine (tertiary amine if the starting material is a secondary amine).



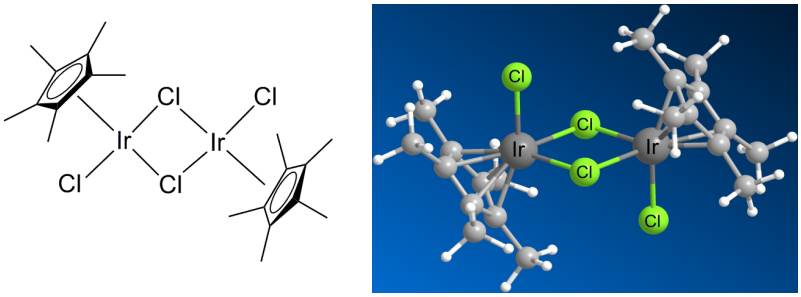
Scheme 2. General pathway of metal catalyzed reaction between alcohols and amines.

The reaction mechanism for N-alkylation of amines with amines has not been proposed with substantial evidence, but it is believed that it proceeds via similar key steps (more details in chapter 2).

1.4.1.1 Iridium

The most commonly used iridium catalyst for N-alkylation of amines with alcohols is $[Cp^*IrCl_2]_2$ (figure 2).[78]

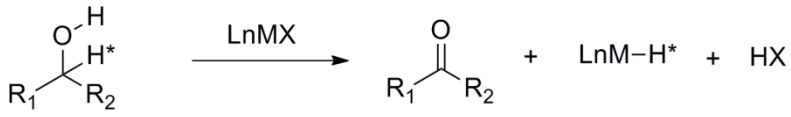
Figure 2. Structure of $[Cp^*IrCl_2]_2$.



This 18 electron iridium dimer has to break down to the 16 electron monomer to be able to coordinate the alcohol. Computational studies have shown that in solution the monomer is slightly favored.[79]

The first part of the mechanism, which involves oxidation of the alcohol to the corresponding aldehyde or ketone, has been studied separately (Oppenauer-type oxidation). Initially, Yamaguchi and co-workers reported the capability of $[Cp^*IrCl_2]_2$ to

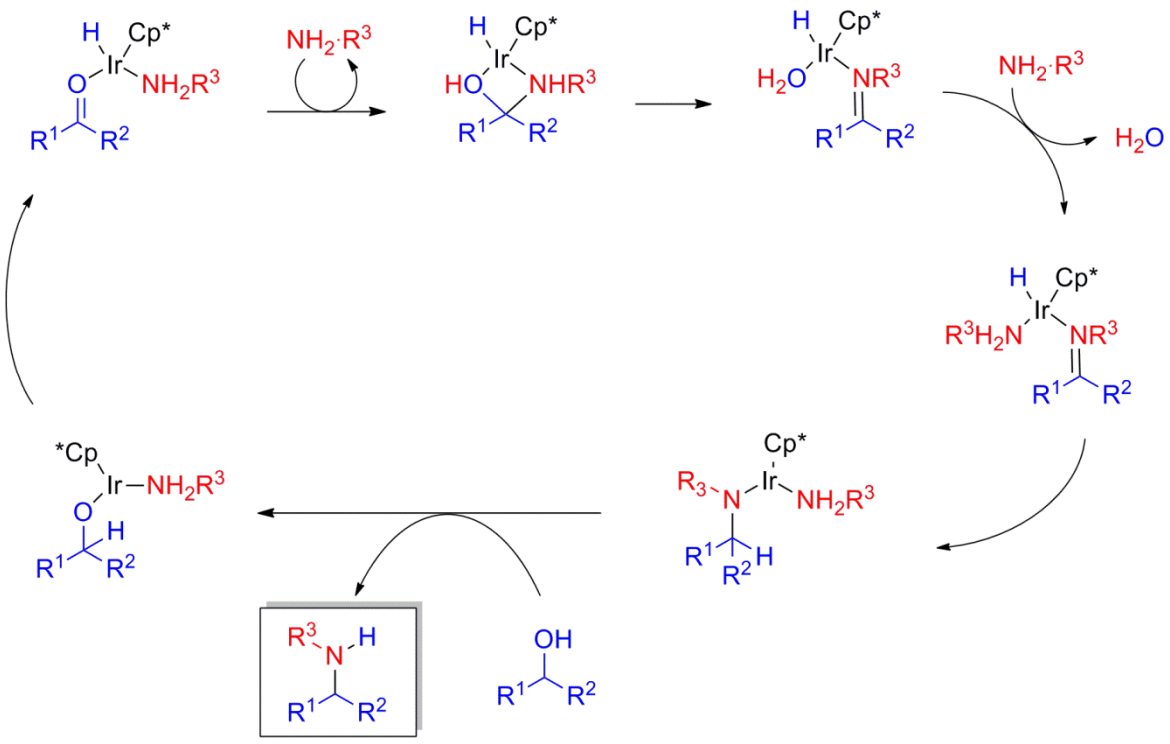
oxidize primary alcohols and benzylic alcohols.[80] The yields of the resulting aldehydes were high and reactions were carried out at room temperature in only 6 hours. However, no mechanistic experiments were conducted and the proposed mechanism was merely a speculation based on work by Bäckvall and co-workers[81,82] regarding a similar reaction with a ruthenium catalyst. More information regarding this oxidation was obtained when Madsen and co-workers performed an experiment developed by Pàmies and Bäckvall.[79] The experiment involved racemization of a deuterated chiral alcohol to determine if a monohydride or a dihydride catalytic species is formed.[83] The product of the reaction contained almost only deuterium, which is indicative of a monohydride mechanism of the oxidation (scheme 3).



Scheme 3. Monohydride mechanism.

At first it was believed that after oxidation of the alcohol the corresponding carbonyl compound was released and in solution it would react with the amine.[78] Interestingly, a combination of recent experimental and computational studies indicates that the formed carbonyl compound does *not* leave the catalyst and imine formation is proposed to proceed via a hemiaminal bond to iridium in a bidentate fashion.[30,79] In addition, also the imine has to be bound to the iridium species to be able to undergo reduction. This is supported by a test reaction where an imine in the presence of a hydrogen donor is not reduced by the catalyst.[30] Furthermore, computational experiments have revealed that all intermediates most likely stay coordinated to iridium throughout the catalytic cycle.

The precise structure the active catalytic species has been studied by computational experiments by Crabtree and co-workers.[84] The results indicate that bidentate carbonate (most often added to the reaction as $NaHCO_3$) lowers the energy barriers and is therefore likely to act as a ligand. In contrast, Madsen and co-workers argue that it is more likely that the amine starting material act as a ligand as this is present in a large excess compared to iridium.[79] The proposed mechanistic cycle illustrated in scheme 4 is a result of calculations based on an amine-iridium species as the active component.[79]



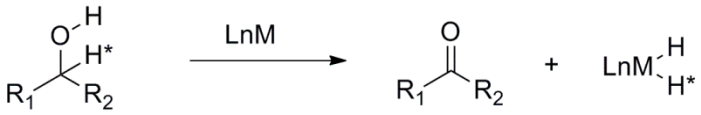
Scheme 4. Proposed reaction mechanism.

The catalytic cycle initiates with coordination of the alcohol to iridium. Subsequent β-hydride elimination furnishes a carbonyl-iridium species. The amine (either internal or external) attacks the carbonyl to afford a hemiaminal and as a result of a proton shift the imine-iridium species is formed. The next step is ligand exchange from water to amine followed by reduction of the imine. Lastly, protonation affords the final product which is then released.

1.4.1.2 Ruthenium

In contrast to the iridium catalyzed reaction where $[Cp^*IrCl_2]_2$ is almost exclusively employed as catalyst, in the ruthenium catalyzed reaction many different ligands are used. One of the most widely used catalytic systems is $RuCl_2(PPh_3)_3$.[85] To the best of our knowledge no general mechanism for the ruthenium catalyzed reactions has been supported with solid evidence as for the iridium catalyzed reaction described above. An example of the importance of the nature of the ligand is the reaction of anilines (or heteroaromatic amines) with alcohols where the dialkylated amines are obtained with $RuCl_2(PPh_3)_3$ and the monoalkylated amine with [Ru(cod)(cot)].[37] Interestingly, modification of $RuCl_2(PPh_3)_3$ to the cationic species $[Ru(PPh_3)_2(CH_3CN)_3]^+[BPh_4]^-$ resulted in formation of only monoalkylated products.[27]

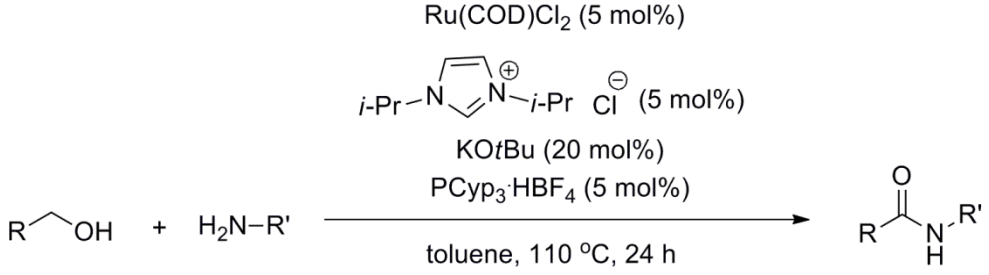
In 1984 Watanabe and co-workers proposed a mechanism based on kinetic measurements of the reaction of aniline and benzyl alcohol catalyzed by $RuCl_2(PPh_3)_3$.[25] The key steps of this were the same as for the iridium catalyzed reaction. However, coordination of intermediates to ruthenium was not investigated. The experiments also revealed that formation of the imine is most likely the rate-determining step. Later Brandt and co-workers used different ruthenium catalysts to determine if the transformation proceeds via a mono- or a dihydride mechanism.[86] The results were to a great extend depending on the exact nature of the ligands. The result of reactions catalyzed by $RuCpCl(PPh_3)_2$ supports the mono-hydride mechanism (as for the iridium catalyzed reaction (scheme 3)), whereas reactions with $RuCl_2(PPh_3)$ seems to occur via a dihydride-mechanism as illustrated in scheme 5.



Scheme 5. Dihydride mechanism.

Williams and co-workers have conducted mechanistic experiments with [Ru(*p*-cymene)Cl_2]$_2$ and DPEphos suggesting that the carbonyl compound can dissociate from ruthenium and that the imine is not necessarily formed while coordinated to ruthenium.[28] This is in contrast to the iridium catalyzed reactions where all intermediates are coordinated throughout the catalytic cycle.[79]

Interestingly, when a ruthenium complex with an N-heterocyclic carbene ligand is employed for coupling of an alcohol and an amine none of the expected amine was formed. Instead, the corresponding amide was formed with extrusion of two equivalents of hydrogen in high yield (scheme 6).[87] The mechanism of the reaction is currently being investigated in the Madsen group.



Scheme 6. Amides from primary alcohols and amines catalyzed by a ruthenium-carbene complex.

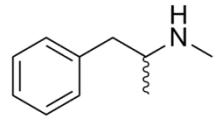
1.5 Concluding remarks

There is a growing awareness of the need to protect the environment in a chemical production. In particular there is a need for new and more environmentally benign synthetic pathways to produce biologically active molecules. N-alkylation of amines with alcohols or amines catalyzed by iridium and ruthenium is a promising method for achieving this goal. In this thesis synthesis of secondary amines and the privileged structures piperazines and indoles from amines and alcohols will be described.

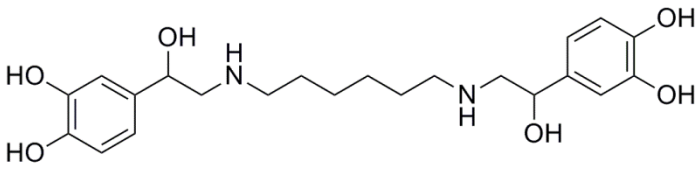
2 Secondary amines

Secondary amines are present in many pharmaceuticals as well as in agrochemicals and fine chemicals.[88] To the best of our knowledge there has not been conducted any survey on the precise number of pharmaceuticals containing secondary amines. However, a 2004 survey of almost 1200 orally administered pharmaceuticals revealed that almost 12% of these contained a (-NH-CH_2-) scaffold.[89] This unit is not necessarily a secondary amine, but it underlines the importance of the core amine function in pharmaceuticals. In figure 3 a few of the many pharmaceuticals containing a secondary amine function are shown.

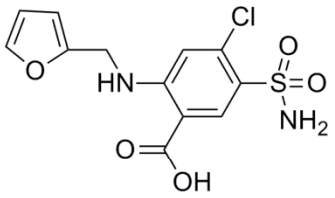
Figure 3. Secondary amine containing pharmaceuticals.



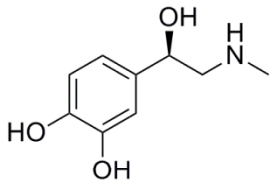
Desoxyn by Abbott (e.g. for ADHD)



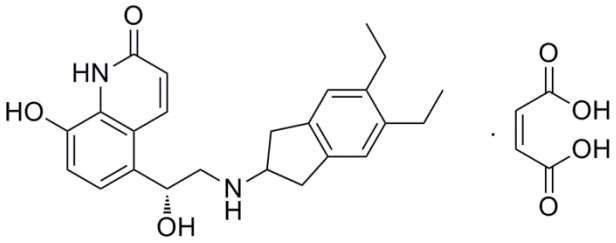
Gynipral by Nycomed (for premature birth)



Lasix by Sanofi-Aventis
(for congestive heart failure and edema)



Epinephrine e.g. by Dey Pharma
(e.g. for cardiac arrest)



Onbrez by Novartis
(for chronic obstructive pulmonary disease)

2.1 Syntheses of secondary amines

As C-N bond formation is one of the most crucial areas in synthetic chemistry in terms of producing biologically active molecules many different syntheses of amines have been developed during the last century. A selection of these methods will be described herein.

The chapter is divided into four parts. The first part describes the progress in N-alkylation of amines with alkyl halides. This is followed by reactions involving reductions (either catalytic or non-catalytic). The last two sections concern N-alkylation of primary amines with alcohols as well as with amines where the focus is on ruthenium and iridium catalysis.

2.1.1 N-alkylation of primary amines with alkyl halides

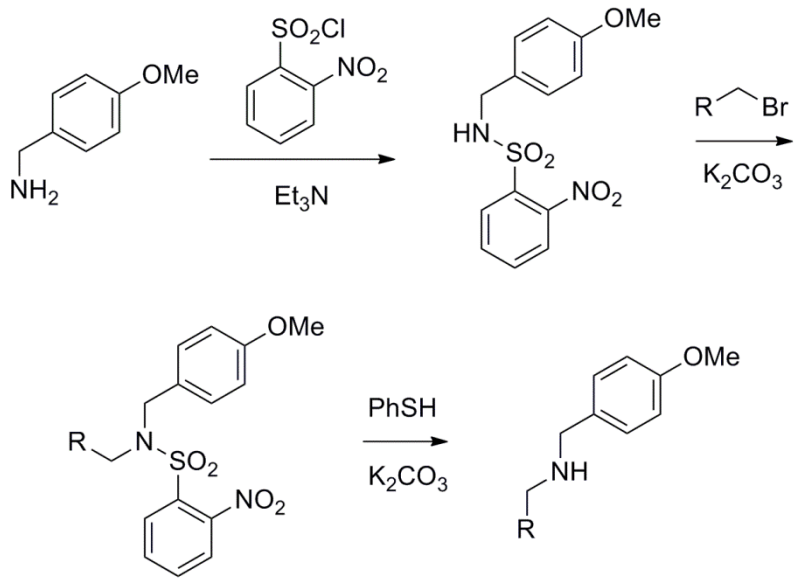
In theory, the simplest reaction for synthesis of secondary amines is N-alkylation of primary amines with alkyl halides. Despite the simplicity it suffers one great disadvantage, which is risk of overalkylation to give both tertiary amines and quaternary ammonium salts. A way to overcome this can be achieved by using a great excess of the primary amine. However, this is not a general solution to overalkylation and it generates large amounts of waste.[88] More promising results are obtained when an excess amount of an inorganic base (e.g. K_2CO_3) is added to the reaction mixture. Hereby, formation of quaternary ammonium salts becomes very unfavorable.[90] Unfortunately, this does not in all cases hamper formation of the tertiary amine as well. An example is the reaction of cyclohexylamine, alkyl bromide and K_2CO_2. The secondary amine is predominantly obtained with 1-bromopentane and the tertiary amine is the sole product when using the shorter alkyl group of 1-bromopropane.[91]

Cesium hydroxide has proved not only to suppress quaternary amine formation but it also hampers overalkylation. For the less active alkyl halides (such as primary and secondary alkyl halides) excess cesium hydroxide is necessary, whereas for the very active halides (such as benzyl and tertiary alkyl halides) a catalytic amount of cesium hydroxide is sufficient.[92]

With the goal to develop an environmentally benign procedure for N-alkylation of amines with alkyl halides Nanda and co-workers have shown that silica gel added to the reaction mixture results in a 5-10 fold excess of the secondary amine to the tertiary amine (in most cases yields are above 75%).[93] This procedure completely avoids the use of excess amine and base as well as addition of other reagents.

A different approach to hamper formation of tertiary amine is introduction of a protection group on nitrogen (scheme 7). Incorporation of this group will make quaternary amine formation impossible and in presence of base the secondary amine is

favored (as explained above).[94,95] The disadvantage is the requirement of additional synthetic steps, resulting in poor atom economy.



Scheme 7. Suppressing tertiary amine formation by introduction of N-protection group.

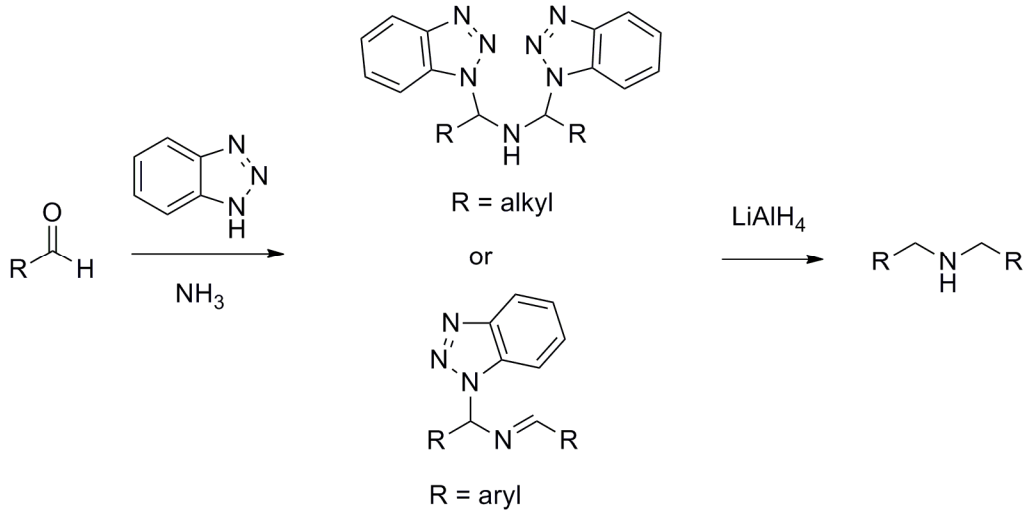
The methods described so far are most efficient with alkyl amines. Reactions of aryl amines often proceed to the tertiary or quaternary amines, hence no general procedures using secondary alkyl and aryl amines are reported.[88] Secondary aromatic amine formation is better achieved utilizing metal catalysis.

In general N-alkylations with alkyl halides are not suitable in large scale productions as they are highly toxic. Furthermore, the reactions have high E-factors, which is both a disadvantage for the environment as well as the cost of the production.

2.1.2 Reduction reactions

2.1.2.1 Reductive amination/alkylation

In "indirect reductive amination" the first step is reaction of a ketone or an aldehyde and an amine to produce an imine. This imine is subsequently reduced to the secondary amine by an appropriate reducing agent (e.g. by $NaBH_4$). As illustrated in scheme 8, ammonia can also be used as the nitrogen source in the reaction with aldehydes and benzotriazoles followed by reduction with $LiAlH_4$.[96] This method provides symmetrical secondary amines.

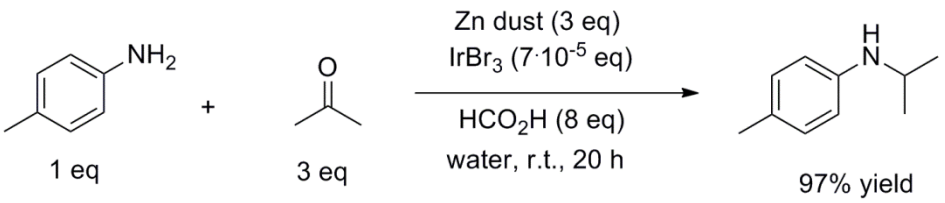


Scheme 8. Ammonia as the nitrogen source in a reductive amination reaction.

To reduce the number of steps, "direct reductive amination" can be applied. In this reaction the amine and the carbonyl compound react in the presence of the reducing agent. Therefore the reducing agent has to be chosen wisely as it must not reduce the starting carbonyl compound. Several efficient reducing agents have been reported and one of the most commonly used agents is $NaCNBH_3$, which was first introduced in 1971.[97] A disadvantage of this reagent is that it is highly toxic and therefore not suitable for large scale synthesis. Reducing agents such as $NaBH(OAc)_3$[98] or a combination of $Ti(O^iPr)_4$ and $NaBH_4$[99] are therefore more suitable. Still, the major disadvantage of reductive amination is the use of a stoichiometric amount of the reducing agent.[88]

2.1.2.2 Catalytic reductions

Bieber and co-workers have shown that reductive alkylation of aliphatic ketones with aryl amines to give secondary amines can be achieved in the presence of zinc and a minute catalytic amount of $IrBr_3$ (scheme 9).[100] The advantage of this reaction is the optimal reaction temperature and it is suitable for a broad range of amines (aryl and benzyl) and ketones (yields range from 75-100%). The disadvantages are the requirement of a great excess of acid and in most cases also excess of ketone is necessary.



Scheme 9. Zinc mediated reductive amination with iridium catalysis.

Other catalytic reductions include:

- Catalytic hydrogenation of imines with various metals (e.g. asymmetric hydrogenation catalyzed by Pd, Rh and Ir).[101]
- Catalytic hydrogenation of secondary amides (e.g. by ruthenium catalysis).[102,103]
- Reductive N-alkylation of nitroarenes with alcohols (e.g. by gold catalysis)[104] or with nitriles (e.g. catalyzed by palladium on carbon, which also works for aliphatic nitro compounds)[105,106].
- Reductive N-alkylation of amines with nitriles (e.g. by palladium or rhodium on carbon under a hydrogen atmosphere).[107]

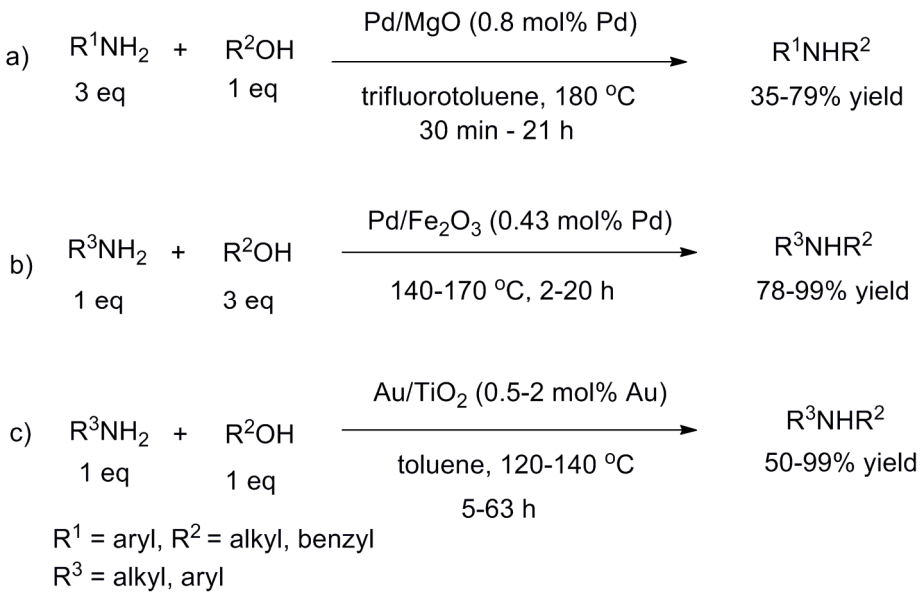
Despite the fact that these reactions are catalytic and therefore minimize waste, they all employ starting materials which are not as readily available as amines and alcohols.

2.1.3 N-alkylation of primary amines with alcohols

As described in chapter 1, N-alkylations of amines with alcohols have received an increased interest during the last three decades. This section is concerned with the progress in this area specifically aimed at syntheses of secondary amines. Not only the ruthenium and iridium catalyzed reactions are described, but also a brief description of catalysis by other metals is included.

2.1.3.1 Palladium and gold

The metal catalyzed N-alkylations of amines have been widely studied, and here some of the more recent reports will be briefly discussed. In scheme 10 three different catalytic systems are shown (all reactions are performed in closed systems).

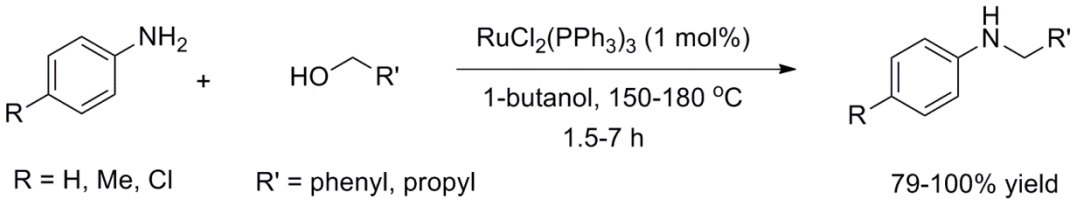


Scheme 10. N-alkylation of amines with alcohols catalyzed by palladium or gold.

In reaction a), palladium supported on magnesium oxide has been employed.[108] The reaction is extremely fast for unsaturated alkyl amines (usually less than 1 hour), whereas the saturated alkyl amines require a much longer reaction time. The disadvantage of the reaction is the necessity of a great excess of the amine. Reaction b) involves a catalytic system consisting of palladium supported on iron oxide.[109] In this method the alcohol has to be added in excess. Reaction a) and b) are both environmentally unfriendly as a result of poor atom economy. Reaction c) uses equimolar amounts of amine and alcohol and the active catalyst is gold nanoparticles.[110] This system is efficient for a broad range of amines and alcohols, though it is best suited for aromatic amines (shorter reaction time and higher yields). In general these heterogeneous reactions often have the advantage of being more practical as the catalysts are easier to recover compared to homogeneous catalysts.[109]

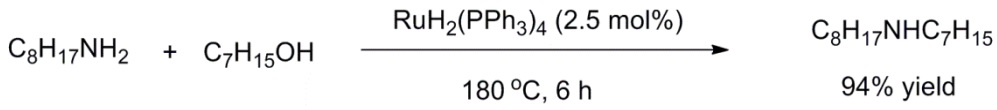
2.1.3.2 Ruthenium

The first publication involving ruthenium catalysis was published in 1981. Anilines and small primary alcohols were condensated to furnish secondary amines in the presence of $RuCl_2(PPh_3)_3$.[18] The yields were relatively low (less than 55%) and aniline had to be used in excess. Interestingly, a few years later the authors published an improved method where equimolar amounts of anilines and alcohol were reacted in presence of the same catalyst to provide the secondary amines in high yields (scheme 11).[25] Even methanol could be employed in the reaction to give N-methylanilines.[26]



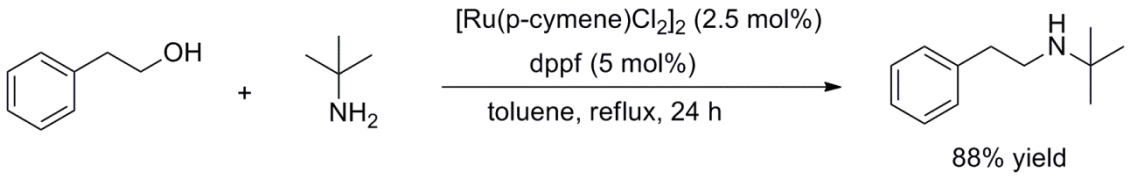
Scheme 11. N-alkylation of arylamines with benzylic or aliphatic alcohols.

Also $RuH_2(PPh_3)_4$ is a very efficient catalyst for reaction of alkyl amines with alkyl alcohols to give aliphatic secondary amines (scheme 12).[38]



Scheme 12. N-alkylation of alkyl amines with alkyl alcohols.

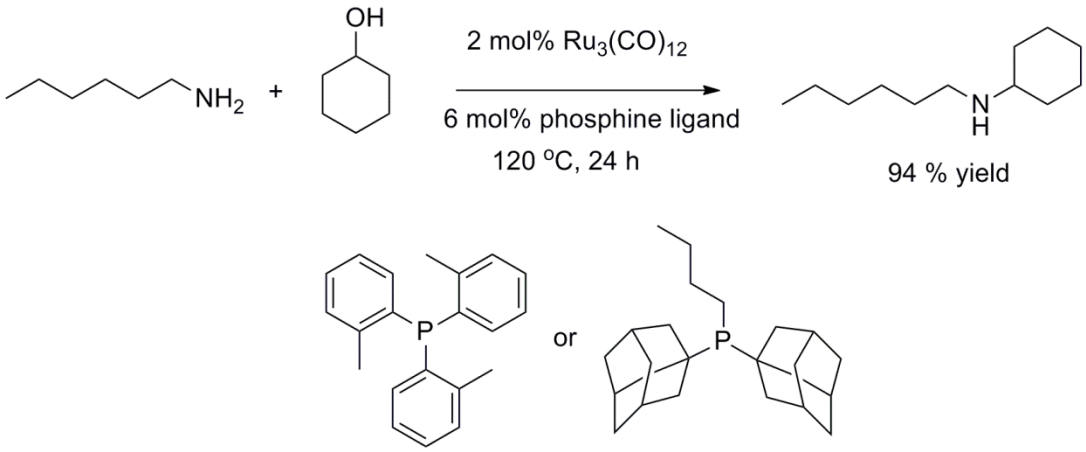
More recent results show that the catalytic system consisting of [Ru(*p*-cymene)Cl_2]$_2$ and dppf is capable of catalyzing the reaction of a primary amine bearing a *tert*-alkyl group (as well as aryl groups) with primary alcohols (scheme 13).[28,39] The reaction affords the secondary amines in high yields (60-93 %).



Scheme 13. Employment of sterically hindered primary amines.

Later, the authors published an improved method where microwave irradiation reduced the reaction time to 1-2 hours and the products were still obtained in the same high yields.[29]

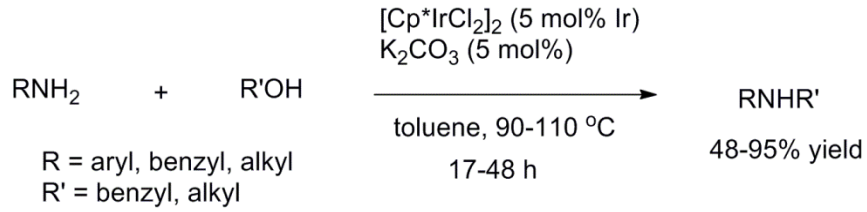
Only few methods utilize secondary alcohols as they are more difficult to oxidize. Beller and co-workers have employed $Ru_3(CO)_{12}$ and this proved to be a very efficient catalyst for employing secondary alcohols in the N-alkylation with aliphatic amines (scheme 14).[42] In most cases the aryl phosphine ligand is superior with yields ranging from 29-92% yield (the lowest yields are when secondary amines are employed).



Scheme 14. Employment of a secondary alcohol.

2.1.3.3 Iridium

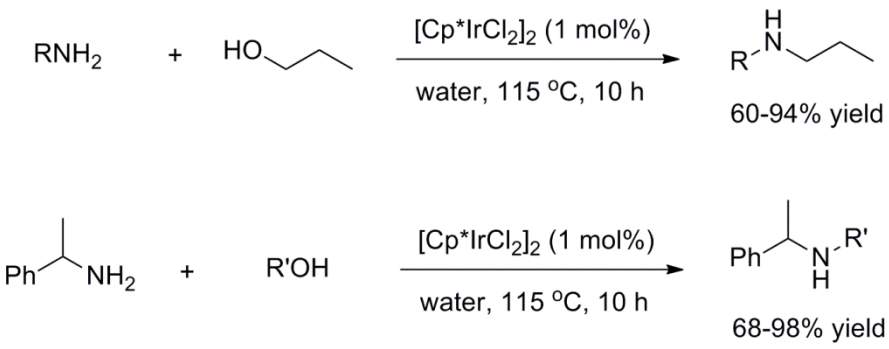
One of the first general methods using iridium catalysis for synthesis of a broad range of secondary amines was published in 2003 (scheme 15).[20] The reaction was most efficient for anilines and benzyl amines, whereas alkyl amines reacted slower and afforded lower yields.



Scheme 15. Iridium catalyzed condensation of amines and alcohols.

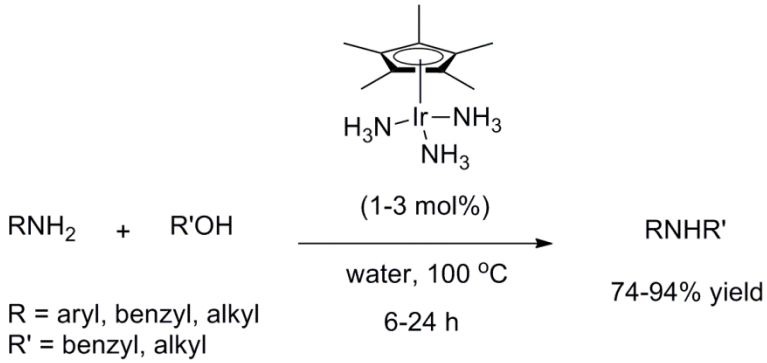
The authors later optimized the reaction conditions. Decreasing the amount of $[Cp^*IrCl_2]_2$ and additive to 1-3 mol% Ir proved to be sufficient to maintain high yields.[30] In some cases the yields were even increased (yields ranged from 71-98 %).

N-alkylation in water was initially reported by Nordstrøm and Madsen in 2007 in the synthesis of piperazines from diols and diamines catalyzed by $[Cp^*IrCl_2]_2$ and $NaHCO_3$ (more details in chapter 3).[111] Based on that work, Williams and co-workers published the corresponding results of N-alkylation of primary/secondary amines with primary/secondary alcohols in 2010 (scheme 16).[49] Noteworthy, additive was not required for the transformation and still the yields were high.



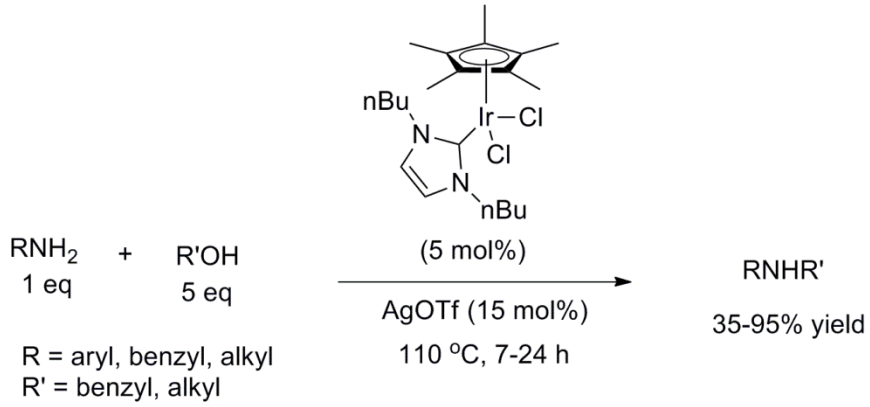
Scheme 16. Use of water as solvent in N-alkylation of amines with alcohols.

Yamaguchi and co-workers have developed a different iridium catalyst that is active in water and a broad range of amines and alcohols have been examined with excellent results (scheme 17).[36] Also in this case no additive is required and additionally, the reaction can be performed under air.



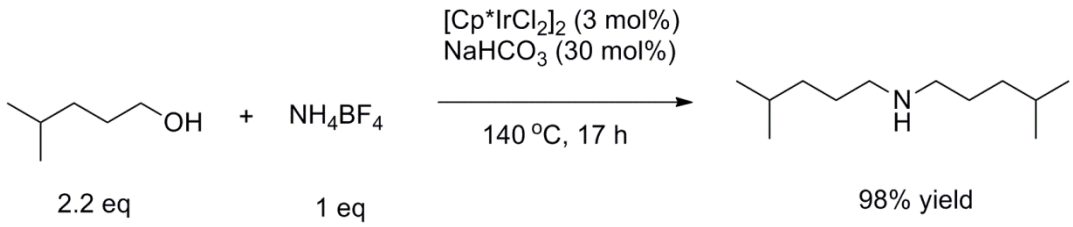
Scheme 17. Use of a water soluble iridium catalyst.

Even carbene ligands are effective in the process. A system consisting of a [$IrCl_2Cp^*$(NHC)] complex together with AgOTf is suitable for the reaction of a broad range of amines and alcohols (scheme 18).[31] Though, in some cases also the corresponding tertiary amine is formed. A major disadvantage is that the alcohol has to be used in a great excess to obtain the moderate to high yields.



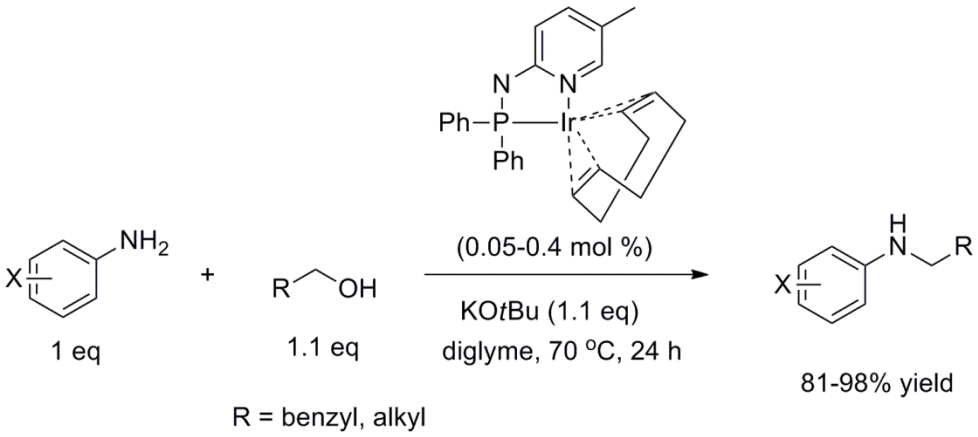
Scheme 18. Use of a carbene ligand for iridium catalysis.

Recently, ammonium salts have been employed as the nitrogen source for the N-alkylation reaction. NH_4BF_4 and a simple primary or secondary alcohol are converted in the presence of $[Cp^*IrCl_2]_2$ to symmetric secondary amines in 50-98% yield (scheme 19).[76] The authors have also used aqueous ammonia and this also furnishes the secondary amines in slightly higher yields.[75] It should be noted that if ammonium acetate is employed the tertiary amines are obtained in equally high yields.[76]



Scheme 19. Reaction between ammonium salt and primary alcohols.

Kempe and co-workers have published several papers utilizing $[IrCl(cod)]_2$ together with P,N-ligands to achieve secondary amines from anilines (or heteroaromatic amines) and primary alcohols.[32-35] The most recent results are illustrated in scheme 20.[35] The reaction affords secondary aryl amines in very high yields. Interestingly, the reaction failed to convert alkyl amines. The major disadvantage is that the stoichiometric amount of base is required. However, what it lacks in preventing waste it gains by a very low catalyst loading as well as a low reaction temperature, which had not been seen before.



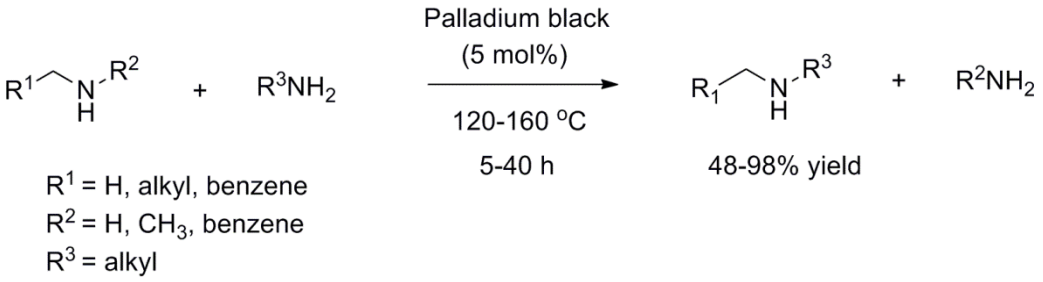
Scheme 20. Reaction between arylamines and primary alcohols catalyzed by an iridium-P,N-ligand complex.

2.1.4 N-alkylation of primary amines with amines

Secondary amines can also be achieved by N-alkylation of amines with primary amines. This section describes the progress in this area specifically aimed at syntheses of secondary amines. Focus is on ruthenium and iridium, but also other metals are briefly discussed.

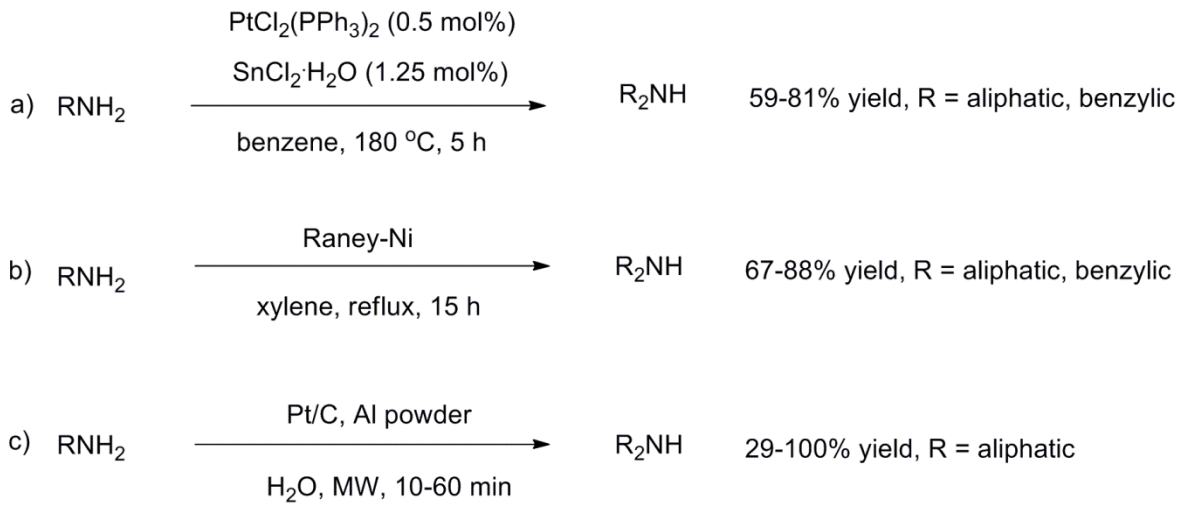
2.1.4.1 Palladium, platinum, nickel

The early attempts to form secondary amines from primary amines in a catalytic manner include alkyl group exchange of primary and secondary amines in the presence of palladium black as illustrated in scheme 21.[112] The reaction suffers from poor atom economy as it also produces a primary amine in an equimolar amount relative to the formed secondary amine.



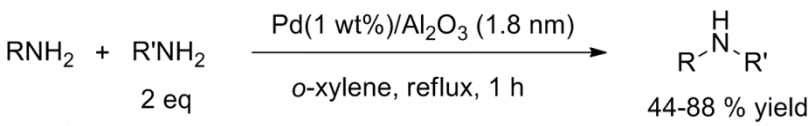
Scheme 21. Use of palladium catalysis for cross-coupling of amines.

To avoid a stoichiometric amount of waste, self-condensations of primary amines are more efficient. It can be achieved by different catalytic systems (scheme 22). In reaction a) $PtCl_2(PPh_3)_2$ combined with $SnCl_2{\cdot}H_2O$ affords secondary amines in moderate to good yields in a short reaction time.[113] Reaction b) involves Raney-nickel in a heterogeneous catalytic system which also proved to be very efficient.[114] More recently water and microwave irradiation has been employed in the reaction. In the presence of palladium on carbon and aluminum powder secondary amines are acheived in an extremely short reaction time (reaction c).[115] However, it suffers from a great variation in yields due to formation of the corresponding tertiary amines (for primary alkyl groups) and the corresponding ketones (for secondary alkyl groups).



Scheme 22. Metal catalyzed self-condensation of primary amines.

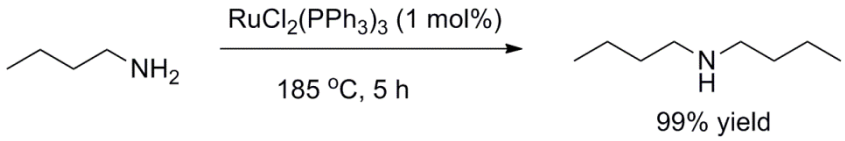
Recently, Shimizu *et al* reported cross-couplings of amines catalyzed by alumina-supported palladium nanoclusters (scheme 23).[116] The majority of the examined reactions involved condensation of a benzylic amine with morpholine (only one example with an aliphatic amine). The major advantage is the good to high yields obtained in a very short reaction time. Additionally, the catalyst has a very high turn over number (even higher than that of $[Cp^*IrCl_2]_2$ in a similar reaction)[117]. However, a disadvantage is the use of excess amount of the secondary amine starting material.



Scheme 23. Cross-coupling of amines catalyzed by palladium nanoclusters.

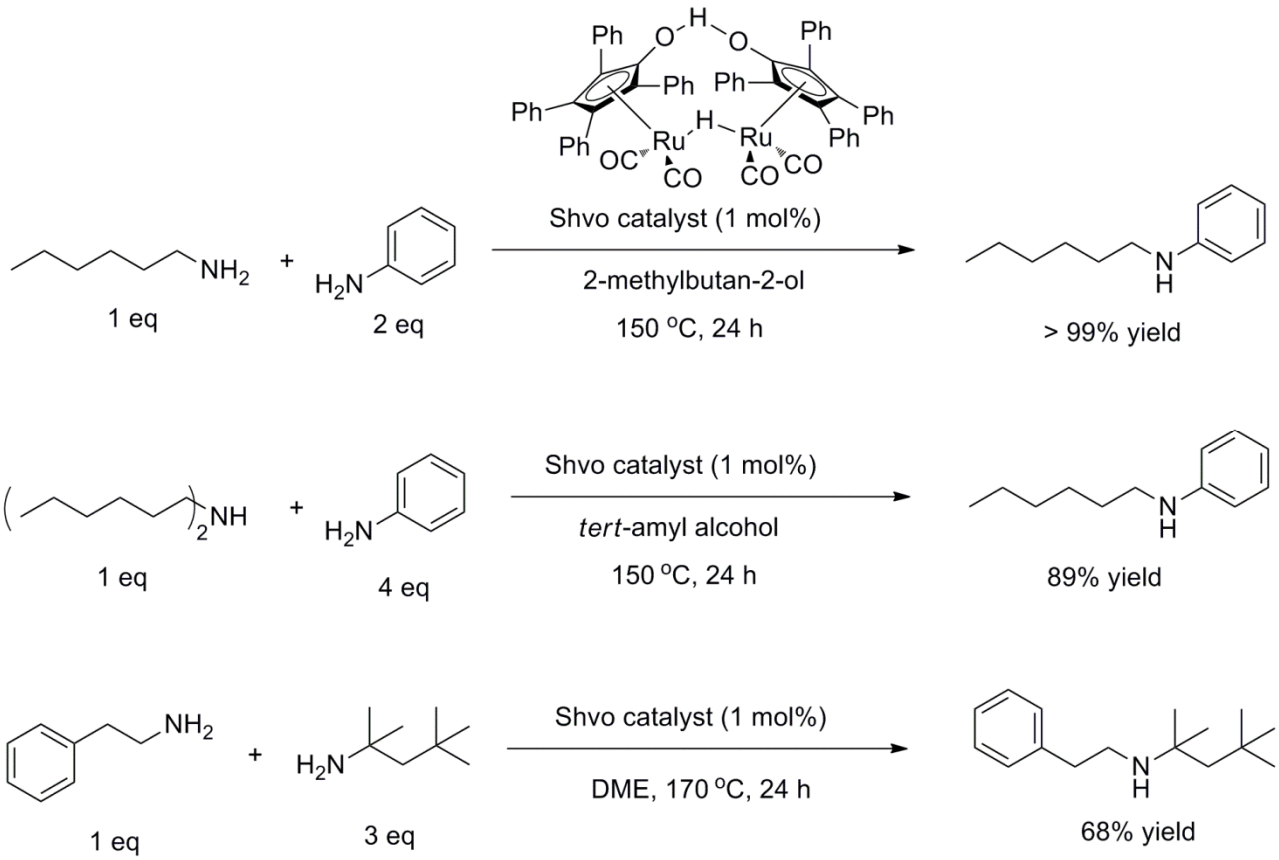
2.1.4.2 Ruthenium

One of the first reports on the use of ruthenium in N-alkylation of amines with amines utilized $RuCl_2(PPh_3)_3$ for self-condensation of simple primary amines to afford the symmetric secondary amines in high yields.[118] This is exemplified with reaction of butylamine in scheme 24. Other aliphatic amines as well as benzylamine afforded the products in 72-99% yield.



Scheme 24. Ruthenium catalyzed self-condensation of a primary amine.

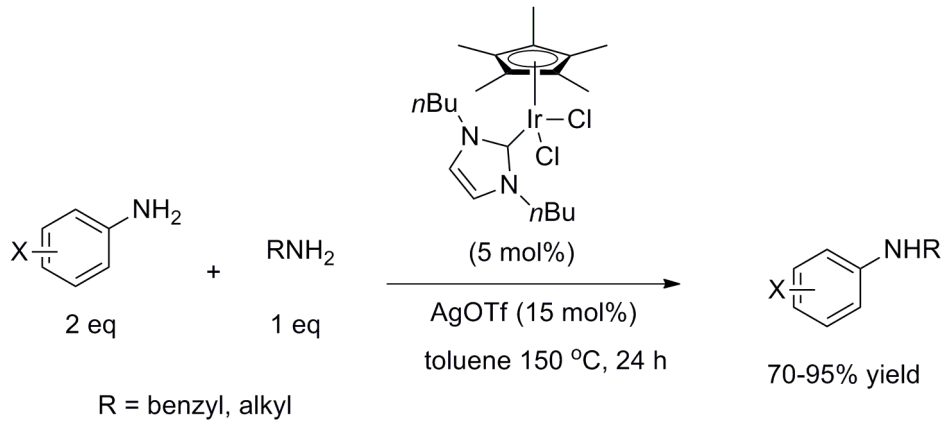
Aryl amines react with alkyl amines in the presence of the Shvo catalyst (scheme 25).[119] The reaction is suitable for a broad range of anilines and the aliphatic amine can have either primary or secondary alkyl groups. Yields range from 76-99%, but unfortunately the aryl amine must be added in excess. As also illustrated in scheme 25 even secondary amines as well as tertiary amines (not shown) can be used in the reaction and still the desired secondary amines are obtained in high yields.[120] However, the reaction requires addition of an even greater excess of aniline and additional waste is generated. The authors have also reported the condensation between two different aliphatic amines in the presence of the Shvo catalyst (also illustrated in scheme 25).[121] One of the amines *must* bear a tertiary alkyl group. Additionally, the reaction temperature is increased and an excess of the *tert*-alkyl amine is required to obtain satisfying yields (49-94 %). To some extend also secondary and tertiary amines can condensate with the *tert*-alkyl amine to give the desired secondary amine.



Scheme 25. Use of the Shvo catalyst for cross-couplings of amines.

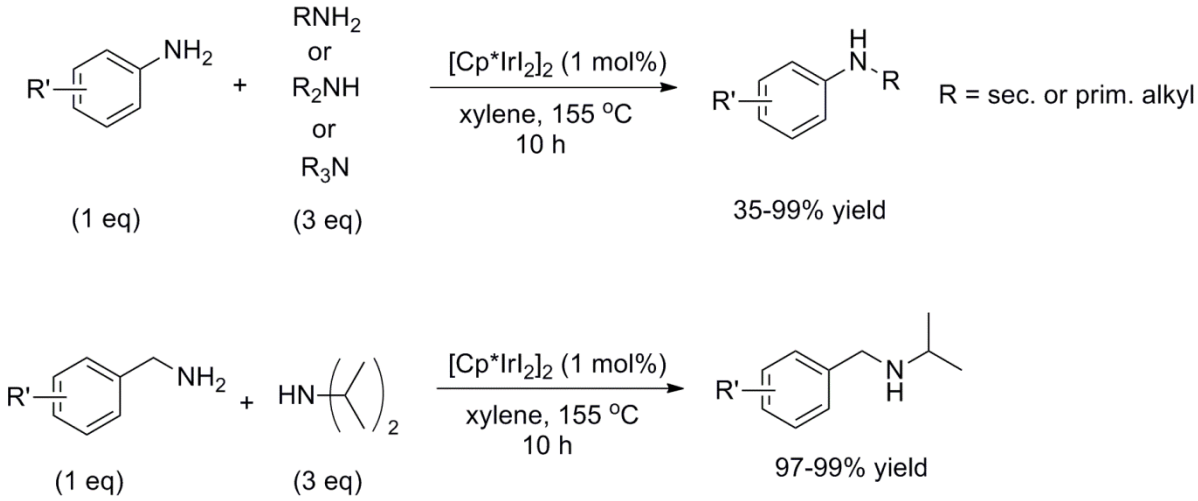
2.1.4.3 Iridium

Iridium has not been as thoroughly explored as a catalyst for N-alkylation of primary amines with amines. Though, one example utilizes an iridium-carbene complex to catalyze the reaction between an aryl amine and a primary amine (scheme 26).[31] The yields are high and the temperature is moderate, but the disadvantage is that the arylamine has to be added in excess.



Scheme 26. Use of a carbene ligand for cross-coupling of amines.

Similar transformations have been performed with $[Cp^*IrI_2]_2$ as the catalyst and both primary, secondary and tertiary amines were able to react with anilines (scheme 27).[117] The bulkier the alkyl group is, the higher the yields are. The most efficient of the examined amines were $(i\text{-Pr})_2NH$ with the majority of the yields being close to quantitative. $(i\text{-Pr})_2NH$ was also reacted with a range of benzyl amines (and alkylamines) with great success. The disadvantage of all the transformations is the use of a great excess of alkylamine.



Scheme 27. Iridium catalyzed cross coupling of amines.

2.1.5 Concluding remarks

It has been illustrated that secondary amines can be synthesized by many different methods. The non-catalytic methods all produce large amounts of waste. Additionally, for N-alkylation with alkyl halides and reactions involving strong reducing agents, these are in particular unfriendly to the environment as these materials are highly toxic. As a result of this, the methods are not suitable for large scale synthesis. Many of the described methods (both catalytic and non-catalytic) have issues with selectivity as also the tertiary amine is formed. More environmentally benign results are obtained by metal catalyzed N-alkylation of primary amines with either alcohols or amines. These methods all have advantages and disadvantages and there is still room for improvements. In particular iridium has not been studied in depth as a catalyst for N-alkylation of amines with amines.

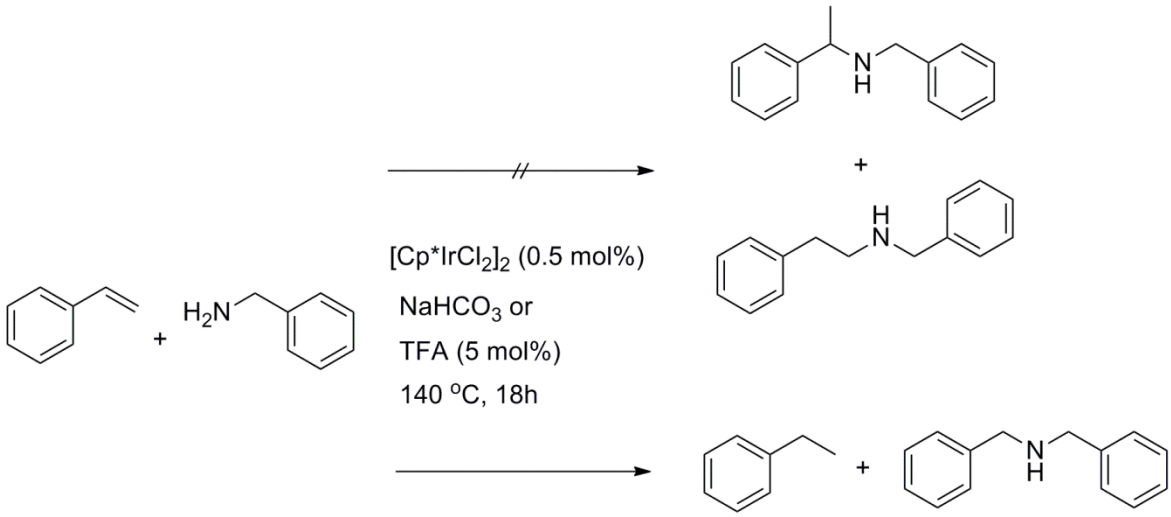
2.2 Aim of the project

A green and atom economical method for synthesis of secondary amines (both symmetrical and unsymmetrical) is desired. In particular it was decided to aim at a simple and straightforward method for isolation of products.

2.3 Results and discussion

2.3.1 N-alkylation of amines with amines

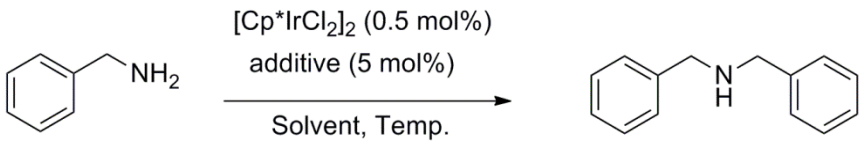
The project initially started with examination of the capability of the catalyst to form C-N bonds. This was tested by reacting benzylamine and styrene with a catalytic amount of base or acid. The system was not able to furnish the desired product(s). Interestingly, it was able to reduce the double bond of styrene and self-condensation of benzylamine to dibenzylamine was observed in trace amounts (according to GC-MS) (scheme 28).



Scheme 28. Initial investigations.

Resultantly, the self-condensation of benzylamine was further examined starting with screening of reaction conditions. The results are summarized in table 7.

Table 7. Self-condensation of benzylamine.



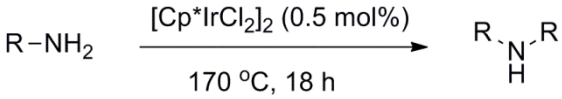
Entry	Solvent	Additive	Temp (°C)	Time(h)	Conversion (%)[a]
1	Toluene	-	140	18	0
2	Toluene	TFA	140	18	<5
3	-	-	140	18	~ 10
4	-	TFA	140	18	~ 10
5	-	-	160	18	~ 90
6	-	-	160	30	100[b]
7	-	-	170	18	100
8	-	-	180	6	~ 90

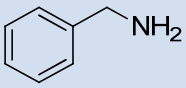
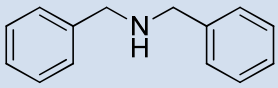
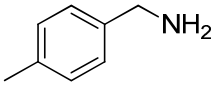
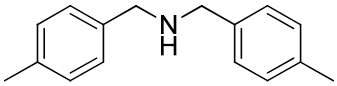
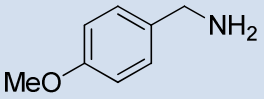
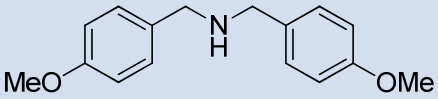
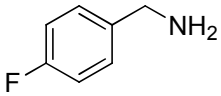
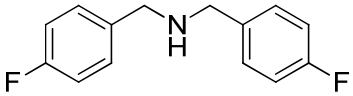
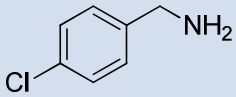
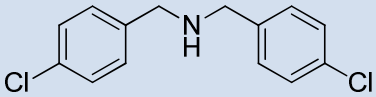
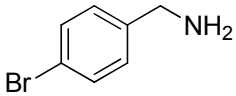
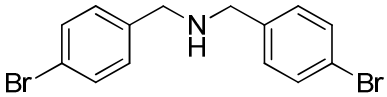
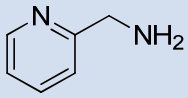
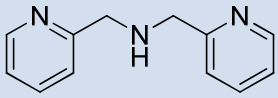
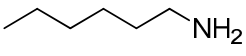
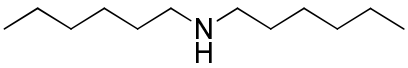
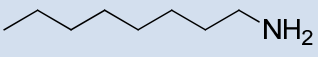
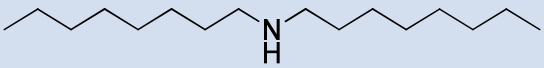
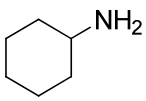
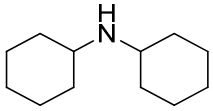
a: by GC-MS. b: in this case also unidentified compounds were observed.

Initially, the reaction was performed in toluene at 140 °C in the absence of an additive, but this gave a very poor conversion (entry 1). Addition of an acid additive did not improve the result significantly (entry 2). In the absence of a solvent the reaction afforded some conversion and dibenzylamine was observed in a trace amount (entry 3). Neither in this case did an acidic additive improve the outcome of the reaction (entry 4). Instead, a higher reaction temperature (160 °C) afforded almost full conversion of benzylamine (entry 5). Increasing the reaction time to 30 hours resulted in complete conversion (entry 6). Unfortunately, the prolonged reaction time increased the formation of by-products. As a compromise a reaction temperature of 170 °C with a reaction time of 18 hours proved successful (entry 7). An attempt to shorten the reaction time to 6 hours by increasing the temperature to 180 °C was, however, not successful as full conversion was not obtained (entry 8).

With the optimized reaction conditions focus was then moved to substrate screening. All products were isolated by direct vacuum distillation from the reaction mixture. The results are summarized in table 8.

Table 8. Self-condensation of primary amines.



Entry	Amine	Product	Yield (%)[a]
1			70[b] (66)
2			75[c]
3			77[c]
4			83
5			80 (62)
6			76
7			79
8			72[d] (49)
9			72[d]
10			73[d] (39)

Yields in parenthesis are after 8 hours. a: isolated yield by distillation. b: 86% yield after 72 hours. c: synthesized by former bachelor student Paw Jensen. d: after 72 hours.

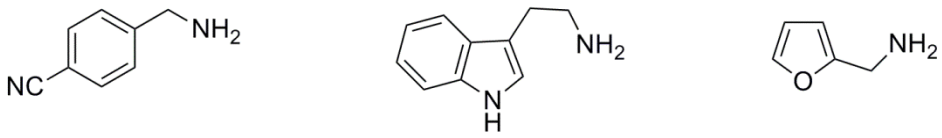
Dibenzylamine was isolated in 70% yield (entry 1). If the reaction time was reduced to 6 hours a yield of 66% was obtained, whereas a reaction time of 72 hours afforded 86% yield. Consequently, it was decided to maintain the reaction time of 18 hours despite the higher yield with a prolonged reaction time. Both methyl and methoxy substituents as well as halogenated benzylamines afforded the dibenzylamines in good yields ranging from 75-80% yield (entry 2-6). The reaction of 4-chlorobenzylamine was also stopped and

purified after 8 hours and this afforded an isolated yield of 62% compared to 80% after 18 hours (entry 5). It is noteworthy that even a bromo substituent is stable under the reaction conditions (entry 6). This serves as a great advantage as the bromo substituent can act in further functionalization of the molecule. The heteroaromatic compound 2-picolylamine could also be self-condensated affording the product in 79% yield (entry 7). For the aliphatic amines, hexyl-, octyl- and cyclohexylamine, good yields were likewise obtained (72-73%, entry 8-10), but an increased reaction time of 72 hours was required. Isolation of the products after 8 hours gave only 49% yield in the case of dihexylamine and 39% yield for dicyclohexylamine.

In some cases the corresponding tertiary amines were observed if the reaction time was prolonged. In one reaction of benzylamine at 170 ^{o}C for 72 hours tribenzylamine was isolated in 37% yield, but this result was unfortunately not reproducible. Another reaction of benzylamine for 72 hours afforded dibenzylamine in 86% yield.

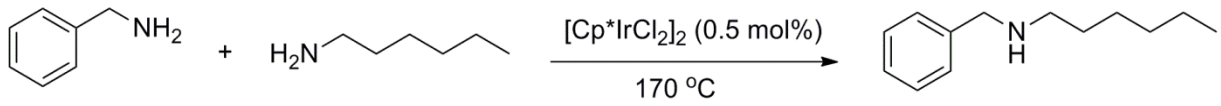
Other substrates tested for self-condensation were 4-cyanobenzylamine, tryptamine and furfurylamine (figure 4). They all decomposed under the reaction conditions.

Figure 4. Failed substrates.



2.3.1.1 Formation of unsymmetrical secondary amine

Attempts to make unsymmetrical amines were conducted with benzylamine and hexylamine (scheme 29). Unfortunately, this was rather unsuccessful as N-hexylbenzylamine was isolated by flash chromatography in only 40% yield. The remainder was dibenzyl- and dihexylamine (not quantified).

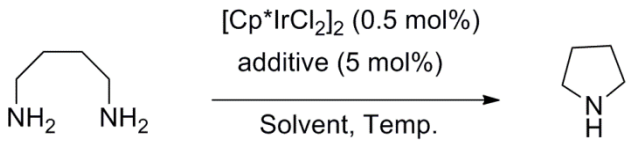


Scheme 29. Cross-coupling of benzylamine and hexylamine.

2.3.1.2 Cyclization of diamines

In 1981 it was shown that cyclic secondary amines could be formed by a self-condensation reaction with a ruthenium catalyst.[118] Therefore it was decided to investigate the cyclization of 1,4-butanediamine to give pyrrolidine. The results are summarized in table 9.

Table 9. Cyclization of 1,4-diaminobutane.



Entry	Additive	Solvent	Temp (°C)	Product (%)[a]
1	TFA	Water	140	< 5
2	$NaHCO_3$	Water	140	< 5
3	TFA	Toluene	140	< 5
4	$NaHCO_3$	Toluene	140	< 5
5	TFA	-	140	< 5
6	TFA	-	160	~ 10
7	TFA	-	140[b]	< 5

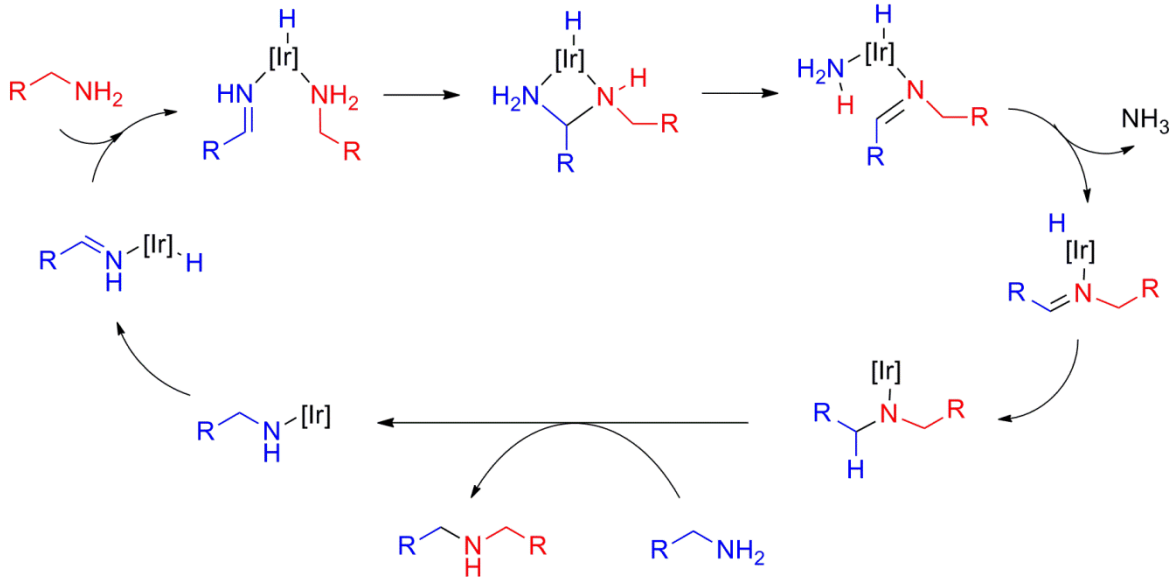
a: by GC-MS. b: after 2 days.

Initially, the reaction was performed in water, but poor conversion and only trace amounts of pyrrolidine was observed with either TFA or $NaHCO_3$ as additive (entry 1 and 2). Changing the solvent to toluene had no effect (entry 3 and 4) and neat reaction conditions did not improve the outcome either (entry 5). Increasing the reaction temperature resulted in slightly more product formation, though also dimerization of the starting material had occurred and still the conversion of starting material was poor (entry 6). Lastly, prolonging the reaction time was examined, but that had no effect on the conversion (entry 7). The one carbon atom longer compound 1,5-pentanediamine was then examined, but also this compound failed at cyclizing and absolutely no piperidine was observed by GC-MS.

As a result of these poor outcomes it was decided that no further attempts to improve the cyclization reaction was conducted. It is clear that the intramolecular transformation is not as straightforward as intermolecular reactions of primary amines.

2.3.2 Mechanism

There was not conducted any experiments in order to determine the reaction mechanism, but a proposal is illustrated in scheme 30. This mechanism is based on the postulated mechanism by Madsen and co-workers[79] with all intermediates coordinated to iridium as described in chapter 1.



Scheme 30. Proposed reaction mechanism for N-alkylation of amines with amines.

Firstly, the primary amine coordinates to iridium and subsequent oxidation afforded the imine. With the imine still coordinated another primary amine coordinates to iridium and this is followed by attack of the amine on the imine to furnish a cyclic intermediate. A proton shift provides the imine and then ammonia is released from the iridium complex. A final protonation leads to the secondary amine product. The exact nature of the active iridium species has not been illustrated. As for N-alkylation of amines with alcohols it is speculated that the primary amine also acts as a ligand. Therefore, it is possible that ammonia is released as a result of ligand exchange with the primary amine due to the higher concentration of primary amine compared to ammonia initially in the reaction. Whether a mono- or dihydride mechanism is involved could potentially be determined by conducting a racemization experiment with a deuterated racemic amine (as described in the chapter 1).

The work described in this chapter was published in *Synthesis* in 2009 simultaneously with the work by Williams and co-workers[117] (described in section 2.1.4.3). They employed the same catalyst but were able synthesize unsymmetrical secondary amines,

in particular N-isopropylbenzylamines. Unfortunately, the reaction was not examined with simple linear primary amines, and the success of the reaction required a 3-fold excess of the alkyl amine. Additionally, the products were purified by column chromatography and this generates large amount of solvent waste. Synthesis of the unsymmetrical amine described in this thesis was obtained by direct distillation from the reaction mixture in only 40% yield, but it must be kept in mind that it was obtained with equimolar amounts of starting materials making this reaction more atom economical.

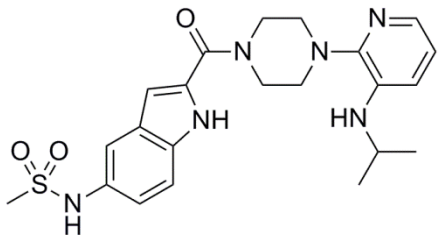
2.4 Conclusion

Symmetrical secondary amines have been synthesized in good yields by a very environmentally benign procedure. No solvent or additives are necessary and products are directly distilled from the reaction mixture. Unfortunately, the reaction conditions were not able to give good selectivity when two different primary amines were mixed. The intramolecular reaction of amines to give cyclic amines was not successful either.

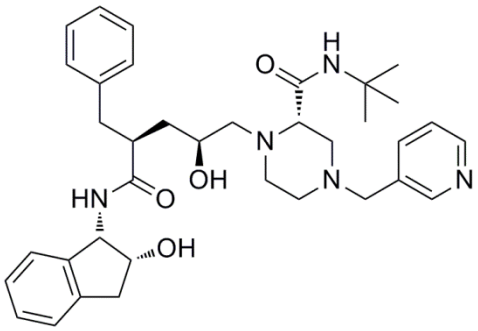
3 Piperazines

A survey of more than 1000 drugs revealed that the piperazine moiety is found in approximately 6% of the orally administered drugs.[89] Due to this relatively high number, there is, also in this field, focus on environmentally benign syntheses. Besides the 4 of the 20 top selling drugs listed in table 2 in chapter 1, a few other examples of piperazine containing pharmaceuticals are shown in figure 5.

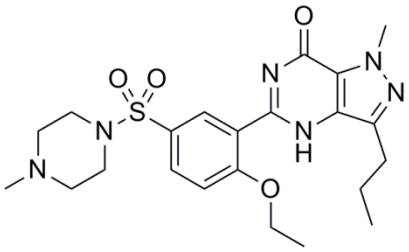
Figure 5. Piperazine containing pharmaceuticals.



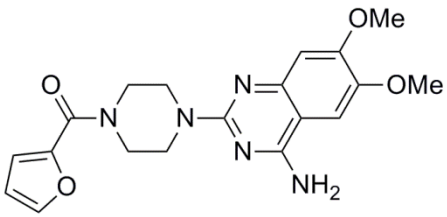
Rescriptor by Pfizer (for HIV)



Indinavir by Merck (for HIV)



Viagra by Pfizer (e.g. for erectile dysfunction)



Prazosin originally by Pfizer
(e.g. for high blood pressure and anxiety)

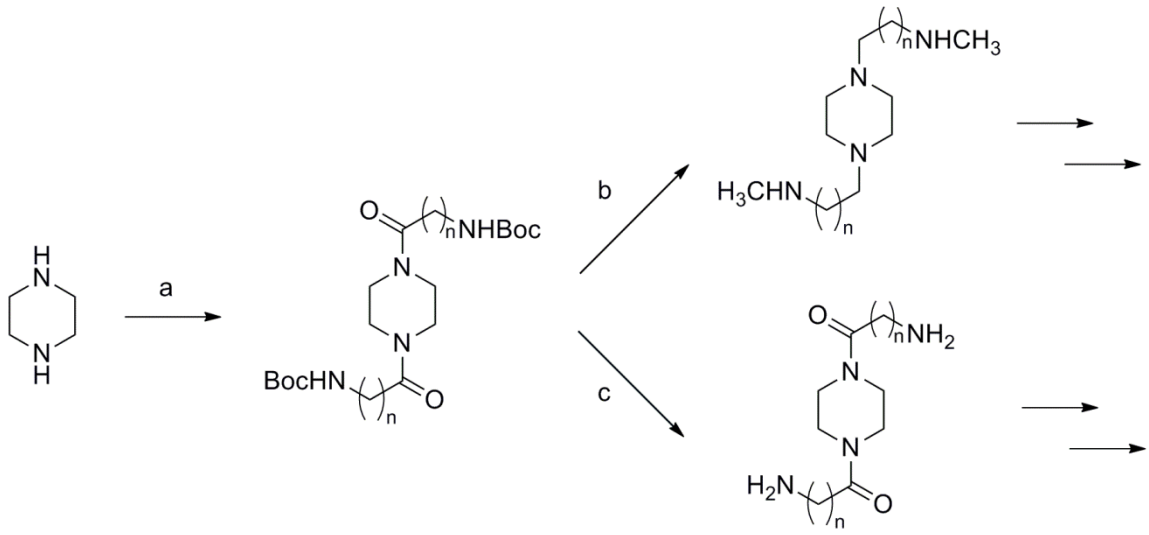
3.1 Methods for synthesis of piperazine derivatives

Piperazine derivatives can have many different substitution patterns. It can have substituents on one or both of the nitrogen atoms and/or one or more substituents on the carbon atoms. Catalytic as well as non-catalytic approaches to synthesize of a wide variety of piperazine derivatives have been published. In this chapter a selection of these methods will be described.

3.1.1 Non-catalytic methods

3.1.1.1 From piperazine (or N-protected piperazine)

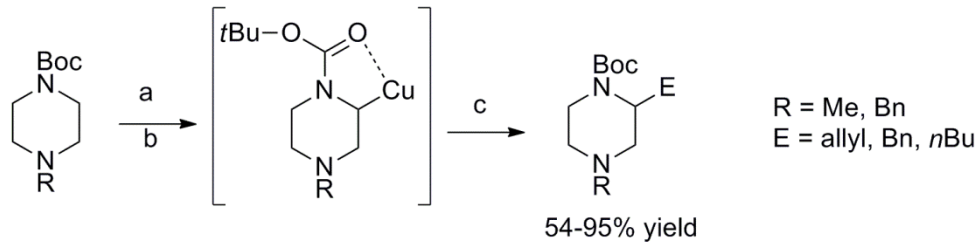
Piperazine itself is commercially available and it is possible to synthesize derivatives directly, e.g. by N-alkylations.[122] A very simple method is a substitution reaction with an appropriate leaving group (such as chloride) to generate N-substituted piperazines. Despite its simplicity, this method suffers major disadvantages such as risk of over alkylation if only monosubstituted piperazine is desired. Additionally, alkyl/benzylhalides are highly toxic and for this reason the method is inappropriate for large scale synthesis (as also explained for the synthesis of secondary amines in chapter 2). A different approach to N-substituted piperazines from piperazine itself is illustrated in scheme 31. Initially, piperazine is acylated with amino acids and then further functionalization is possible.[123] If only mono-N-substitution is desired the reaction must start with protection of one of the two nitrogen atoms.



(a) N-Boc-amino acid, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, Et_3N, DCM, r.t.; (b) $LiAlH_4$/THF, reflux, N_2; (c) TFA, $CHCl_3$

Scheme 31. Piperazine as starting material for synthesis of N,N'-disubstituted piperazines.

2-Substituted piperazines can be synthesized from Boc-protected piperazine (scheme 32).[124] The reaction proceeds through an initial α-lithiation and this is followed by transmetallation to copper. Subsequently, substitution with electrophilic reagents such as alkyl-, allyl- or benzylbromides takes place. Depending on the added electrophile further functionalization is possible. The procedure suffers from disadvantages such as the use of toxic reagents and the formation of additional waste in the form of the copper species.

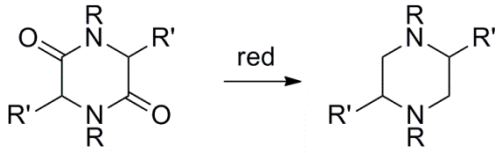


(a) TMEDA, *sec*-BuLi, -78 °C, Et_2O, warm to -10 °C, 1 h (b) CuCN·2LiCl, -78 °C, warm to -50 °C, 30 min (c) electrophile, -60 °C, 1 h, warm to r.t., o.n.

Scheme 32. Synthesis of 2-Substituted piperazines from N,N'-diprotected piperazine.

3.1.1.2 From (di)ketopiperazines

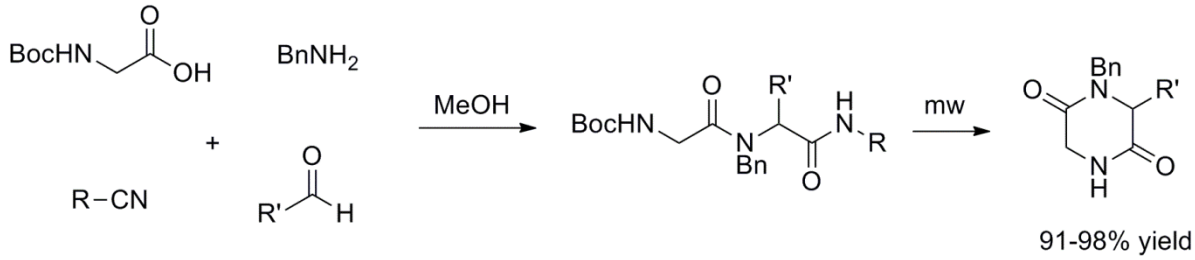
2,6-Diketopiperazines are easily reduced to the corresponding piperazine e.g. by BH_3[125] or $LiAlH_4$[126] (scheme 33). The only requirement for the reduction agent is that it must not affect the substituents on neither carbon nor nitrogen atoms.



Scheme 33. Reduction of diketopiperazines.

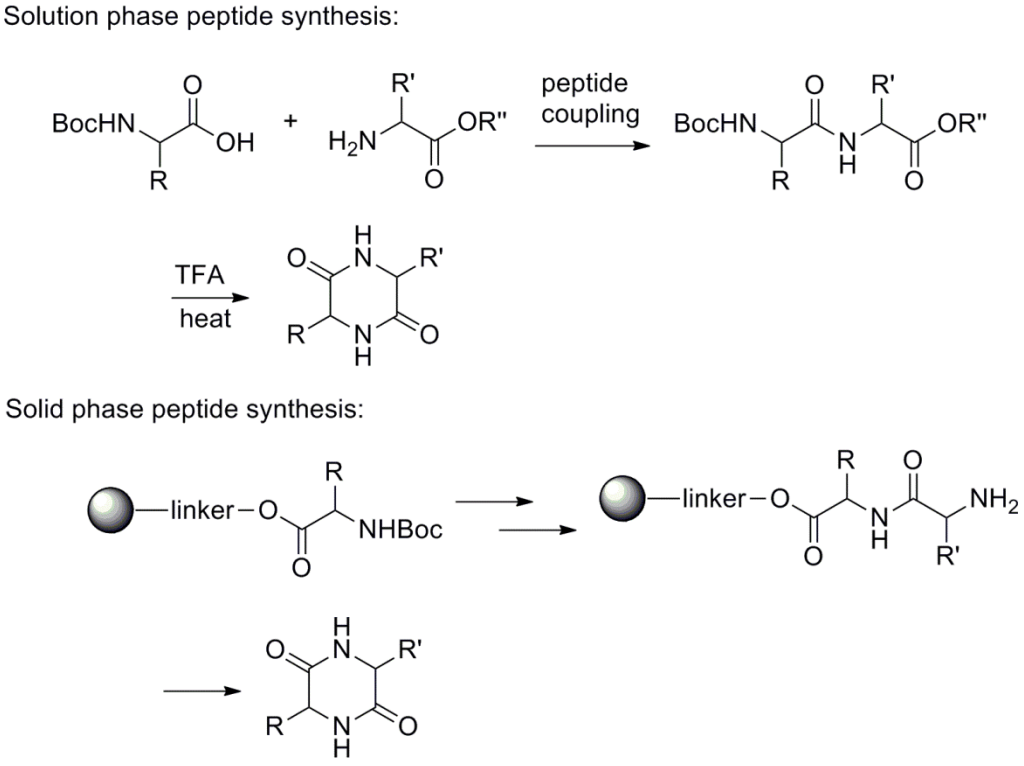
Synthesis of diketopiperazines has been studied for decades. In particular syntheses starting from amino acids (or derivatives) have been reported.[125] For that reason, most of the methods described in this section are based on amino acids.

The Ugi reaction can be used to synthesize 2,6-diketopiperazines. As exemplified in scheme 34 the reaction involves a multi-component reaction (MCR) of four reactants.[126] By using N-Boc protected glycine a simple C,N-disubstituted diketopiperazine is acheived. If other amino acids are used a fully substituted diketopiperazine is obtained.[127] The limitation of the Ugi reaction is the availability of the four reactants.



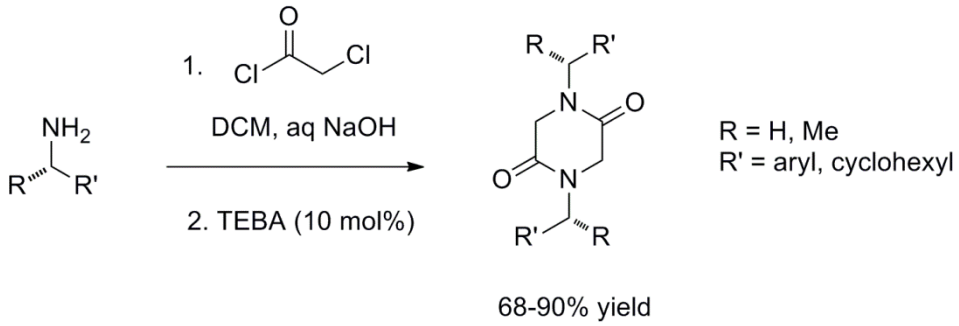
Scheme 34. Diketopiperazines via the Ugi reaction.

Formation of diketopiperazines via peptide synthesis has been widely studied and with great success.[128,129] Examples of both solution phase and solid phase syntheses are illustrated in scheme 35. The solution phase approach involves standard peptide synthesis and when the Boc-group is removed intramolecular peptide formation affords the six-membered ring.[130] The nature of the R/R' groups are limited to those found in the amino acids (unless unnaturally occurring amino acids are used). More commonly solid phase synthesis is applied and also this follows standard procedures.[131,132]



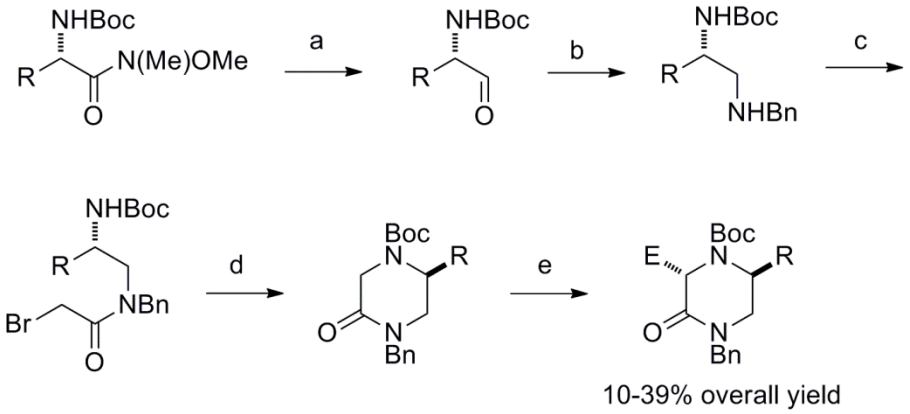
Scheme 35. Peptide syntheses of diketopiperazines.

Symmetrical N-substituted diketopiperazines can be synthesized in one-pot from amines and chloroacetyl chloride in high yields (scheme 36).[133] Despite the simplicity of the reaction, the major disadvantage is the use of the toxic halogenated starting materials.



Scheme 36. One-pot synthesis of diketopiperazines.

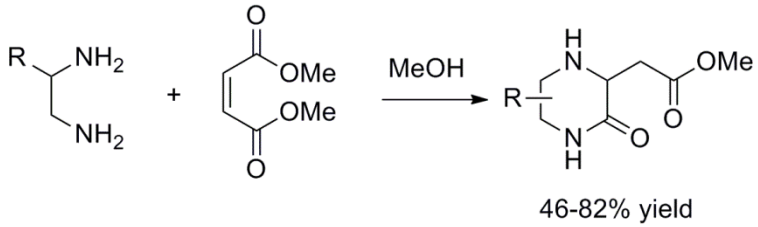
2-Oxo-piperazines can also be reduced to piperazines by $LiAlH_4$.[134] Donati and co-workers have published a method starting from a Weinreb amide (derived from an amino acid) as illustrated in scheme 37.[135] Initially, the amide is reduced to the corresponding aldehyde, which then undergoes a reductive amination. The formed amine is amidated and finally an intramolecular substitution reaction provides the 2-oxopiperazine. If desired, an electrophile can be introduced via *ortho*-lithiation.



(a) $LiAlH_4$; (b) $BnNH_2$, $NaBH_3CN$, CH_3COOH/MeOH; (c) $BrCH_2COOH$, DCC, NMM;
(d) NaH; (e) HMPA, *t*-BuLi, electrophile

Scheme 37. Synthesis of 2-oxopiperazines.

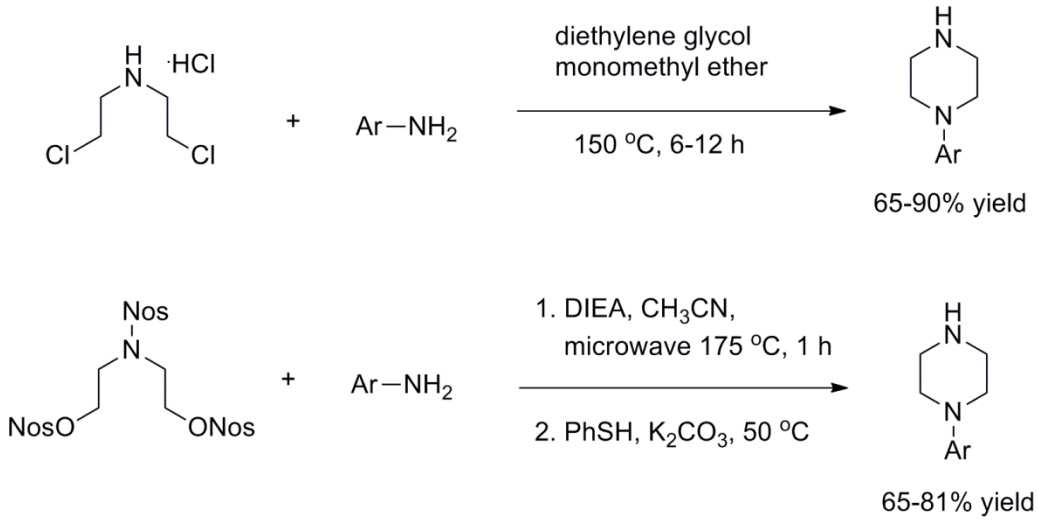
C-substituted ethylenediamine in reaction with dimethyl maleate (or fumarate) is a one-pot approach to 2-oxipiperazines (scheme 38).[136] The limitation of this reaction is the nature of the diamine, as only small substituents are tolerated. Additionally, substitution on nitrogen is possible. However, if the substituent on nitrogen is large the reaction is only successful with carbon unsubstituted ethylenediamine.



Scheme 38. One-pot synthesis of 2-oxopiperazines.

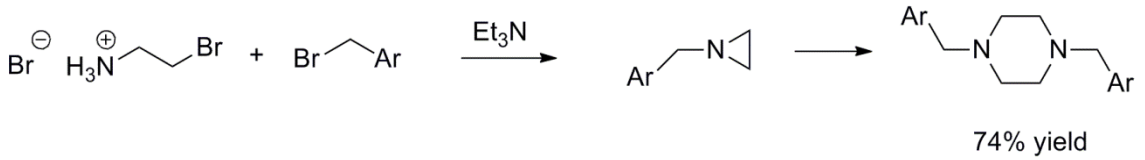
3.1.1.3 By other methods

Bis-(2-chloroethyl)amine hydrochloride is reacted with aryl amines to provide N-aryl substituted piperazines as illustrated in scheme 39.[137] The advantage of the procedure is the good atom economy, as HCl is the only by-product and no additives are used. However, the reaction is limited to non-sterically hindered aryl amines. This can be circumvented by the use of a more recent method involving microwave irridation (scheme 39).[138] The drawback is the use of large excess of the base as well as a deprotection step (of N-Nos). In addition, the procedure suffers from poor atom economy.



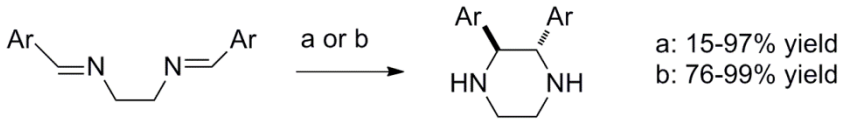
Scheme 39. N-arylsubstituted piperazines from bis-(2-chloroethyl)amine hydrochloride.

Symmetrical N-substituted piperazines are the product of the reaction between ethylamine hydrobromide and benzyl bromides (scheme 40).[139] The disadvantage is the use of excess amounts of the base as well as the use of the toxic halogenated starting materials.

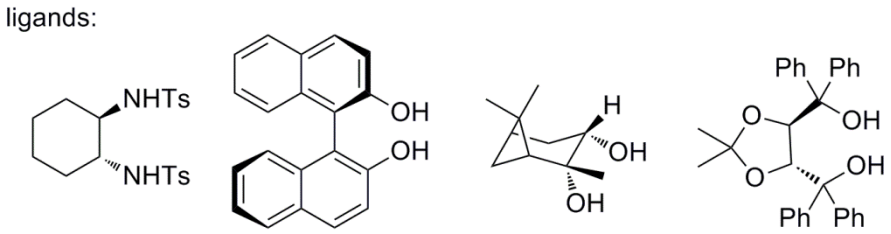


Scheme 40. Synthesis of symmetrical N-substituted piperazines.

An example of the synthesis of C-substituted piperazines from diimines is illustrated in scheme 41. It is achieved via a cyclization reaction involving intramolecular reductive coupling of the diimines.[140-142] The drawback of this reaction is the use of excess reagents as well as the limited availability of the diimines.

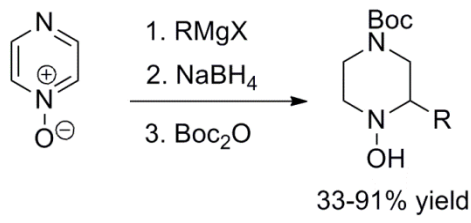


a: 5 eq Zn(0), 2.5. eq ligand, 2.2 eq $TiCl_4$ or $Ti(OiPr)_2Cl_2$ or $Ti(OiPr)_4$, DCM, -70 to 25 °C
b: 1.5 eq Mn(0), 3 eq Brønsted acid, MeCN/Toluene, rt, 4-6 h



Scheme 41. C-substituted piperazines from diimines.

Grignard reagents can be reacted with pyrazine N-oxides to generate N-hydroxylpiperazine, which can then undergo reaction at one or both nitrogen atoms (scheme 42).[143,144] This strategy affords therefore a good handle for further functionalization of the compound. The disadvantage is the use of excess Grignard reagent as well as a reduction step that generates additional waste.

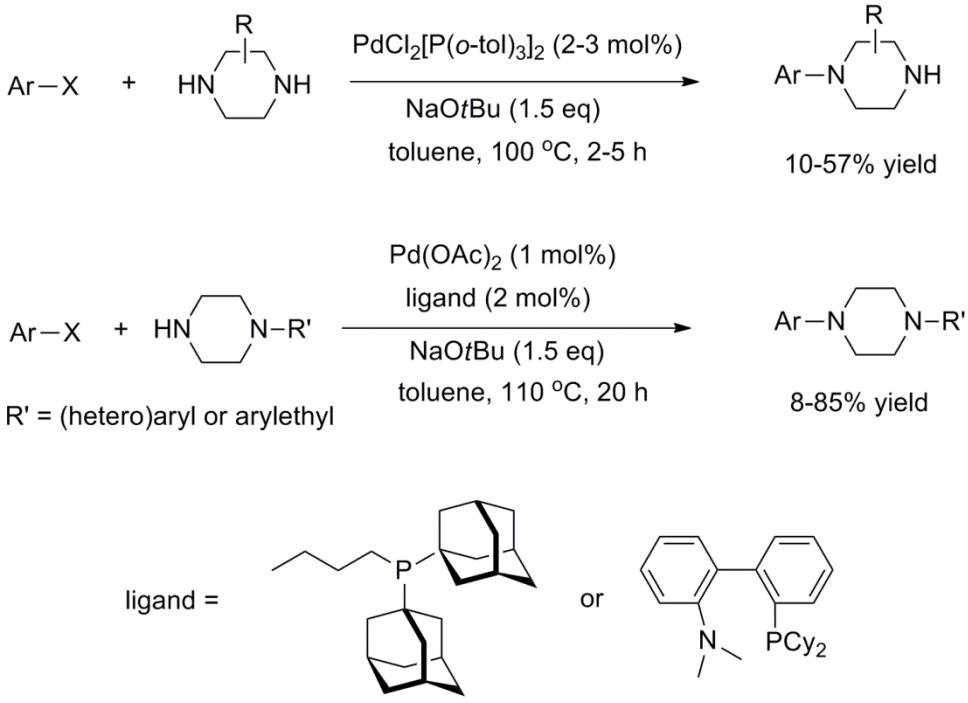


Scheme 42. Use of Grignard reagents and pyrazine N-oxides.

3.1.2 Catalytic methods

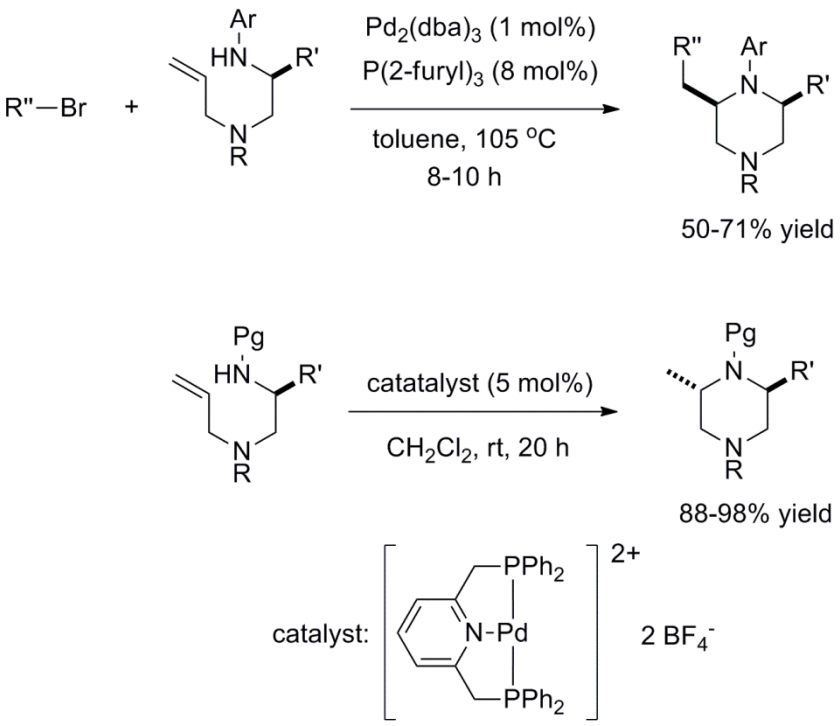
3.1.2.1 Palladium

Palladium is capable of catalyzing N-arylation of piperazines as illustrated in scheme 43.[145,146] The reactions require excess amounts of base and the yields vary tremendously, though the method using $Pd(OAc)_2$ is generally superior.



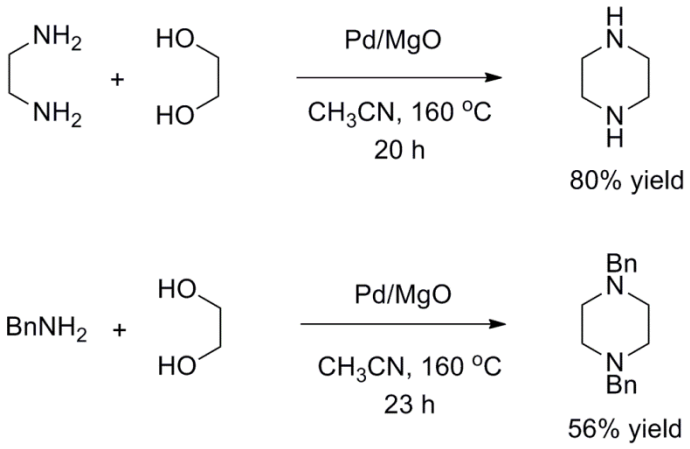
Scheme 43. Palladium catalyzed N-arylation of piperazines.

Another approach to piperazines by palladium catalysis is the intramolecular carboamination reaction[147] and the hydroamination reaction[148] illustrated in scheme 44. The disadvantage for both procedures is the prior synthesis of the starting materials.



Scheme 44. Palladium catalyzed intramolecular formation of piperazines.

Heterogeneous catalysis can also be employed for piperazine formation (scheme 45). When palladium is immobilized on magnesium oxide it is efficient as a catalyst for N-alkylation of amines with alcohols.[108] In addition to synthesis of piperazine itself, also substituted diols and diamines can be utilized in the reaction affording both C- and N-substituted piperazines. The benefit of the reaction is that the catalyst is used in very small quantities (0.8 % Pd) and an additive is not required.

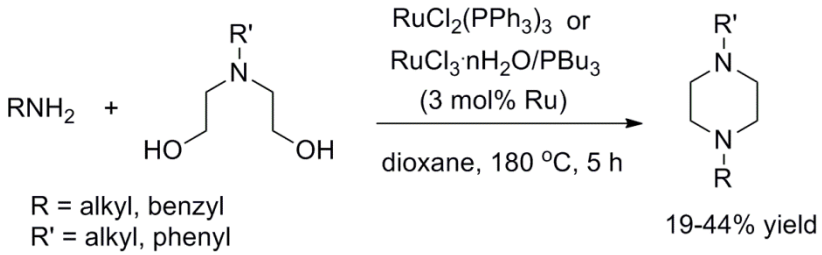


Scheme 45. Use of heterogeneous palladium catalysis for piperazine formation.

3.1.2.2 Ruthenium

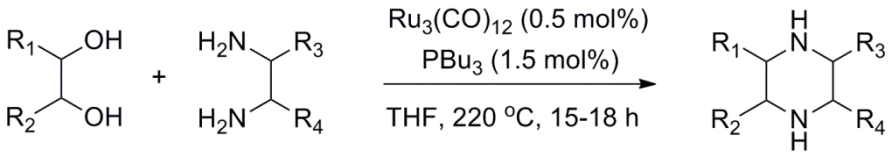
To the best of our knowledge there have been no reports on any general ruthenium catalyzed methods to produce a broad range of both C- and N-substituted piperazines. Some of the methods described in chapter 2 (synthesis of secondary amines) have piperazine as an example, though they do not report synthesis of a broad range of derivatives. In this section some of the methods for synthesis of either C- or N-substituted piperazines will be described.

Reaction between N-substituted diethanolamine and a primary amine in the presence of a ruthenium catalyst afforded N,N'-disubstituted piperazines in moderate yields (scheme 46).[55] The reaction was later examined by van Koten and co-workers with a much more complex ruthenium catalyst.[56] However, the obtained yields were only slightly higher and only two examples were reported.



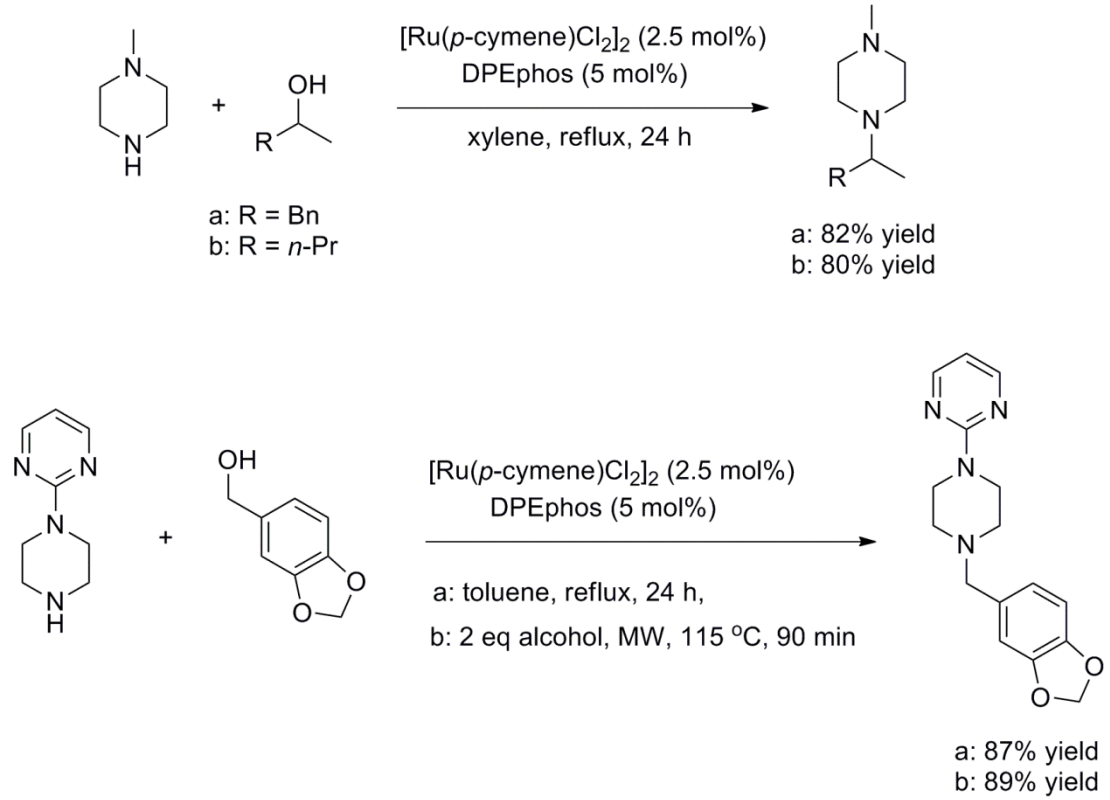
Scheme 46. Ruthenium catalyzed formation of N,N'-disubstituted piperazines.

Jenner and Bitsi have used the borrowing hydrogen methodology to synthesize piperazines from 1,2-diamines and 1,2-diols (scheme 47).[57] The reaction was performed at a very high pressure and only very simple derivatives have been synthesized with a maximum of two substituents; methyl groups or a cyclohexyl group. The reactions afforded full conversion of amines, except for the cyclohexyl substituent where only 75-88% conversion of amine was obtained. Selectivity towards the piperazine ranged from 60 to 100%. Unfortunately, the yields were not reported. Nevertheless, if it is assumed that ethyleneglycol conversion is 100%, the yields range from 60 to 100%.



Scheme 47. Ruthenium catalyzed N-alkylation of amines with alcohols.

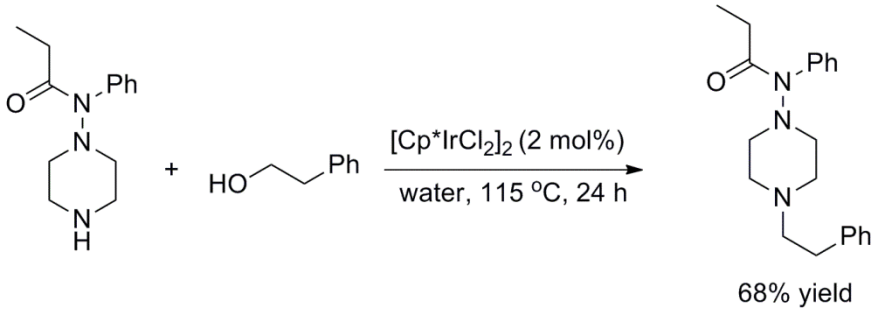
Williams and co-workers have very briefly examined N-substitution of piperazines with a secondary alcohol (in two examples) and with a primary alcohol (in one example) all in high yields (scheme 48).[28] The latter has also been examined under microwave conditions.[29] This proved to be successful as the same high yield was obtained in a highly reduced reaction time.



Scheme 48. Ruthenium catalyzed N-alkylation of piperazine with secondary alcohols.

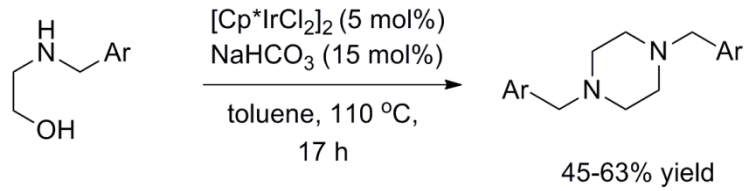
3.1.2.3 Iridium

Methods involving iridium catalysis have not received much attention for piperazine synthesis. Nevertheless, a few examples are worth to be mentioned herein. Piperazine itself can be used as the nitrogen source for the N-alkylation of amines with alcohols as illustrated in scheme 49.[49] Unfortunately, only one specific example has been reported, but the procedure should be applicable with a broad range of alcohols.



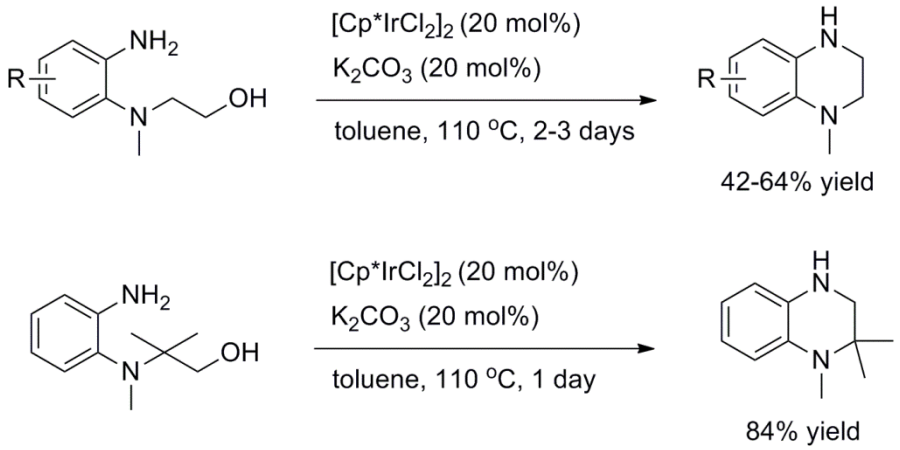
Scheme 49. Iridium catalyzed N-alkylation of piperazine with a primary alcohol.

Yamaguchi and co-workers published in 2009 their work on synthesis of symmetrical N-substituted piperazines from N-substituted ethanolamines (scheme 50).[58] This method is also expected to be successful with C-substituents on the starting material.



Scheme 50. Iridium catalyzed formation of symmetrical piperazines.

Not technically a piperazine, but still an interesting approach, tetrahydroquinoxalines have been synthesized by an intramolecular reaction of arylamino alcohols (scheme 51).[59] The reaction tolerated a quaternary center closest to nitrogen and the corresponding product was isolated in high yield after reduced reaction time (1 versus 2-3 days). This is an interesting result as it is usually difficult to form piperazine derivatives containing a quaternary center.[59] In general the reaction required a relatively high catalyst loading and the methyl substitution on nitrogen was crucial. With hydrogen or benzyl substitution on nitrogen the reaction afforded very low yields.

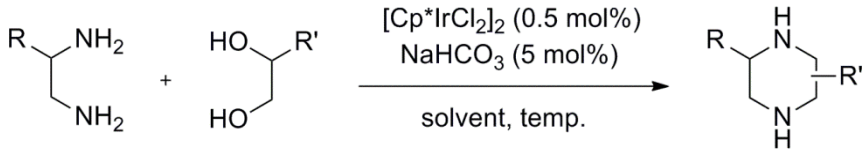


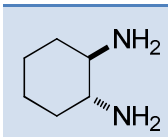
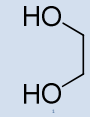
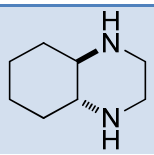
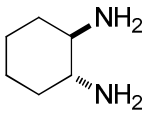
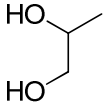
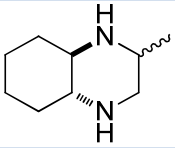
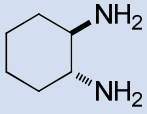
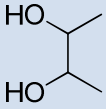
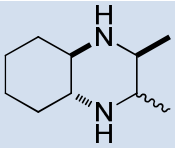
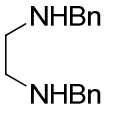
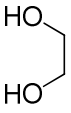
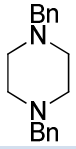
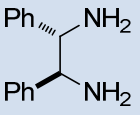
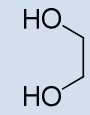
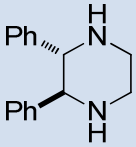
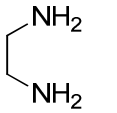
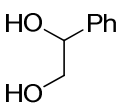
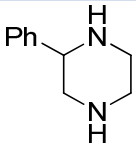
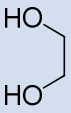
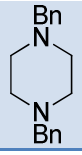
Scheme 51. Iridium catalyzed synthesis of quinoxalines.

3.1.3 Previous work on synthesis of piperazines within the group

In 2007 former PhD student Lars Ulrik Rubæk Nordstrøm published together with Professor Robert Madsen their work concerning iridium catalyzed synthesis of piperazine derivatives.[111] The goal was to condensate 1,2-diamines with 1,2-dialcohol. Several N- and C-substituted piperazines were obtained in good to excellent yields as shown in table 10.

Table 10. Condensation of diamines and diols.

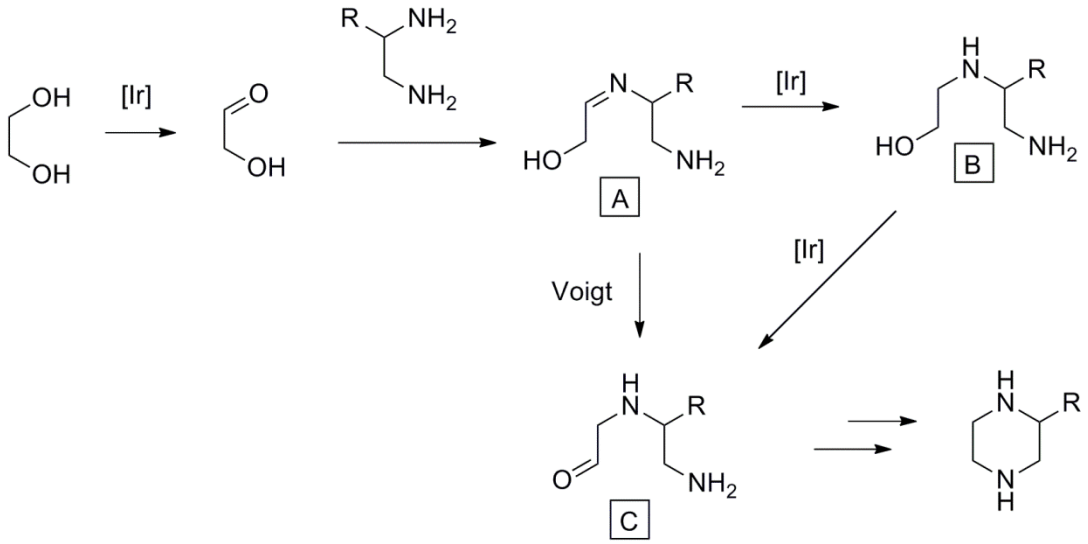


Amine	Diol	Solvent	Temp. (°C)	Product	Yield(%)
		Water Toluene	100 110		96 94
		Water Toluene	100 110		98 87
		Water Toluene	140 140		81 79
		Water Toluene	140 140		73 74
		Water Toluene	100 110		60/86 54
		Water	120		quant.
$BnNH_2$		Neat	160		94

This was the first published method where water is used as a solvent for reaction of amines and alcohols by the borrowing hydrogen methodology. It was a breakthrough as the reaction proceeds via imines which are usually unstable in water. Interestingly the isolated yields are generally high.

It is believed that the reaction proceeds via the mechanism illustrated in scheme 52. Initial oxidation of the alcohol affords the aldehyde (or ketone), which is subsequently condensated with the diamine to form imine **A**. This imine can be reduced by iridium to give amino alcohol **B** and subsequently oxidized to aldehyde **C**. On the other hand, it is

also possible for the imine to undergo a Voigt reaction (isomerization of α-imino alcohols to α-amino ketones)[149,150] to produce aldehyde **C**. This aldehyde undergoes an intramolecular condensation to provide the final product.



Scheme 52. Proposed pathways for piperazine formation from 1,2-diols and 1,2-diamines.

3.1.4 Concluding remarks

It has been illustrated that piperazines can be synthesized by many different methods. The conclusion is similar to that given in chapter 2 concerning synthesis of secondary amines; non-catalytic procedures are not optimal due to large amounts of waste, whereas the catalytic methods show more promising results. The field of metal catalyzed reactions has not been widely explored. In particular in regards to condensation of diols and diamines by iridium catalysis the reaction should be further explored.

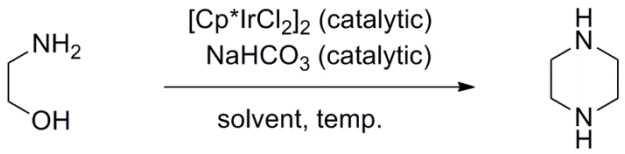
3.2 Aim of the project

C-N bond formation by metal catalysis is a field that has received much attention. However, in terms of piperazine synthesis the number of publications is limited. The work described in this chapter is a continuation of the work by former PhD student Lars Ulrik Nordstrøm. It is desirable to expand the substrate scope to establish the limitations of the reaction. In particular the selectivity of the reaction when both C-substituted diols and C-substituted diamines are condensated is of interest. Attempts to use other types of starting materials (ethanolamine, aromatic amines and ammonium salts) are also explored. As mentioned, $[Cp^*IrCl_2]_2$ is relatively expensive, and it would be of great interest to the industry if a cheaper catalyst could perform the same transformations. Lastly, the mechanism has so far not been supported by any real evidence and should be studied in further details.

3.3 Results and discussion

3.3.1 Piperazine from ethanolamine

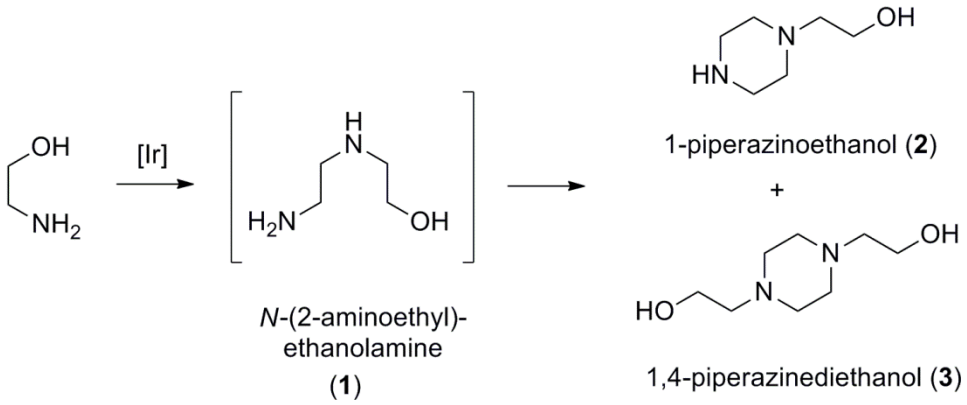
As it is possible to condensate 1,2-diamines with 1,2-diols to give piperazine derivatives it was envisioned that ethanolamine in a self condensation reaction would afford the parent piperazine (scheme 53).



Scheme 53. Piperazine from ethanolamine.

The reaction was tested in a large number of experiments with variations in catalyst loading, temperature, solvent and reaction time; 0.01-1 mol% $[Cp^*IrCl_2]_2$ and 5 mol% $NaHCO_3$ at temperatures ranging from 55 to 110 ^oC in THF, DME, dioxane, toluene or water for 5-24 hours. Unfortunately, all reactions afforded poor conversions and only trace amounts of piperazine were observed. In the pursuit to obtain full conversion only water and neat reaction conditions were further examined and the results are summarized in table 11.

Table 11. Self-condensation of ethanolamine.



Entry	mol% cat	Solvent	Temp (°C)	Time	Conversion (%)[a]	Products[a]
1	0.5	Water or neat	140	1 day	< 10	**1**, **2**, and **3**
2	0.25	water	140	4 days	100	**2** (30%), **3** (27%)
3	0.25	Neat	140	2 days	100	**2** (23%), **3** (25%)
4	0.25	Neat	140	6 days	100	**2** (14%), **3** (20%)
5	0.25	Neat	160	2 days	100	No products

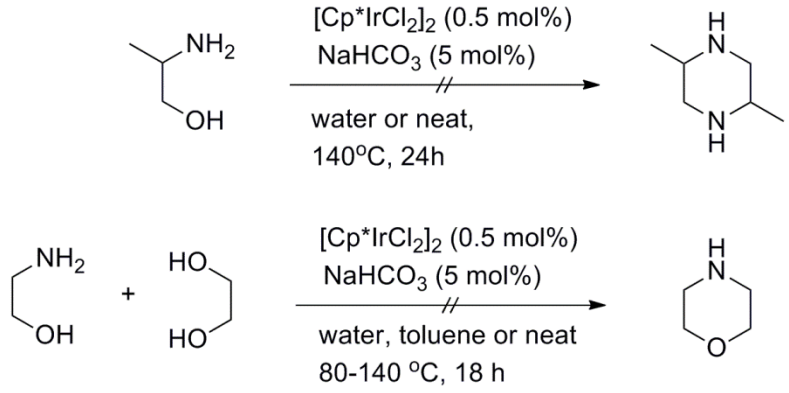
Numbers in parenthesis are isolated yields. a: by GC-MS.

A reaction time of 1 day at 140 °C in water or neat reaction conditions did not afford full conversion (entry 1). Nevertheless, several products were detected by GC-MS analysis; two major products as well as an intermediate. According to the mass of the intermediate it is likely to be *N*-(2-aminoethyl)ethanolamine, which is an expected intermediate in the formation of piperazine from ethanolamine. Unfortunately, the structure has not been confirmed as the compound was not isolated. Extending the reaction time afforded full conversions and the two major products (according to GC-MS analysis) were isolated and identified as 1-piperazinoethanol and 1,4-piperazinediethanol. At full conversion of starting material the intermediate was no longer observed by GC-MS analysis and interestingly still no piperazine was detected. When water was used as solvent a reaction time of 4 days was required to obtain full conversion and this resulted in isolated yields of 30% and 27% respectively, of 1-piperazinoethanol and 1,4-piperazinediethanol (entry 2). With neat reaction conditions a reaction time of 2 days was sufficient, though the yields were slightly lower; 23% and 25% of 1-piperazinoethanol and 1,4-piperazinediethanol, respectively (entry 3). When the reaction time was further increased to 6 days (neat reaction conditions) the yields dropped to 14% and 20% of 1-piperazinoethanol and 1,4-piperazinediethanol, respectively (entry 4). Reaction at 160 °C (neat conditions) resulted in no products according to GC-MS analysis (entry 5). This could be due to polymerization

or possible degradation of the starting material. In no cases were any other compounds isolated. It is possible that the reactions have formed higher polymers that cannot be isolated by column chromatography and are not detectable in GC-MS analysis.

Unfortunately, the ratio between the two products cannot be controlled and the products have a limited possibility for functionalization. This combined with the assumed polymerization of starting material and/or intermediates resulted in the decision not to attempt any further optimization of the reaction conditions.

As illustrated in scheme 54 other strategies were also examined. Attempts to synthesize a piperazine derivative from substituted ethanolamine were briefly investigated. Using 2-aminopropanol instead of ethanolamine at 140 ^{o}C in either water or neat conditions failed completely at affording 2,5-dimethylpiperazine. A change in reactant from using solely ethanolamine to reacting equimolar amounts of ethanolamine and ethylene glycol afforded only trace amounts of morpholine.



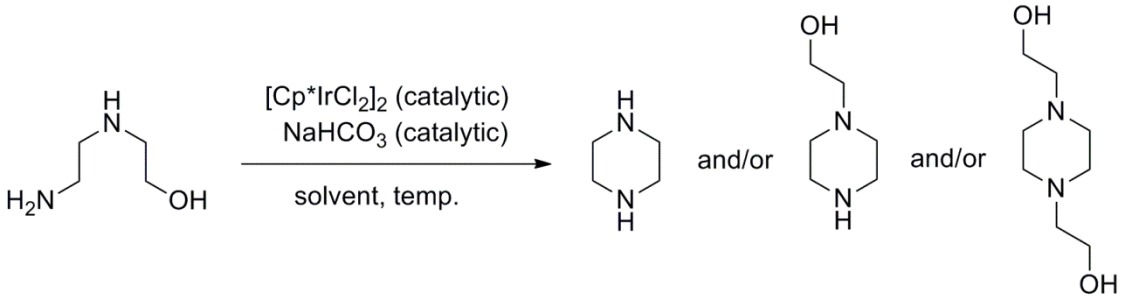
Scheme 54. Failed reactions with ethanolamines.

It is concluded that ethanolamine (and methyl-C-substituted ethanolamine) are not suitable starting materials for piperazine synthesis. Interestingly, Yamaguchi and co-workers had success with self-condensation of N-benzyl ethanolamines to furnish the corresponding disubstituted piperazine.[58] It is possible that the presence of a substituent on nitrogen is crucial - at least it hampers polymerization. It should also be noted that a very high catalyst loading (20 mol%) was used, which might also account for the high yields.

3.3.1.1 Reactions of N-(2-aminoethyl)ethanolamine

As already mentioned, the intermediate observed in the reaction of ethanolamine was not isolated, but on the basis of GC-MS analysis it is possible that it is *N*-(2-aminoethyl)ethanolamine. Despite the failed attempt to synthesize piperazine from ethanolamine it was tested if *N*-(2-aminoethyl)ethanolamine could be an intermediate in the formation of piperazine and/or 1-piperazinoethanol and 1,4-piperazinediethanol. The results are summarized in table 12.

Table 12. Reaction of *N*-(2-aminoethyl)ethanolamine.



Entry	mol% cat	Solvent	Temp (°C)	Time	Products[a]
1	0.25	Neat	140	1 day	piperazine (14% isolated yield)[b]
2	0.25	Neat	160	1 day	Piperazine (31% isolated yield)[b]
3	0.25	Neat	160	2 days	Piperazine (34% GC-yield)[b,c]
4	0.5	Water	140	1 days	Piperazine (70% GC-yield)
5	0.5	Water	140	2 days	Piperazine (100% GC-yield)
6	0.25	Water	140	2 days	Piperazine salt (79% isolated yield)

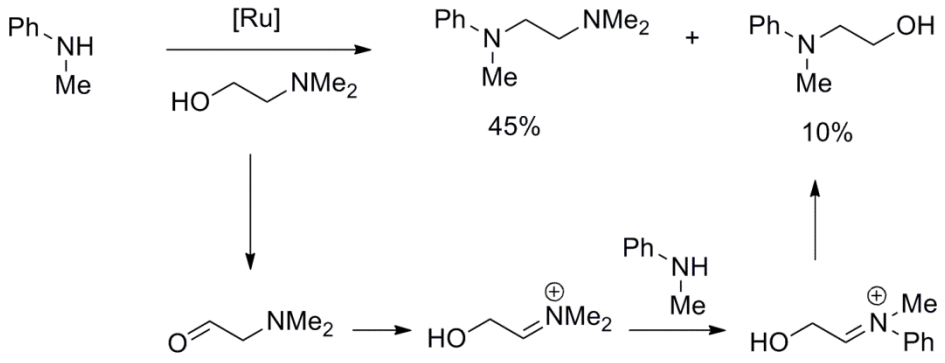
a: by GC-MS. b: 1-piperazinoethanol also observed (not quantified). c: also many minor peaks were observed by GC-MS.

After reaction for 1 day at 140 °C a mixture of starting material, piperazine and 1-piperazinoethanol was observed, and piperazine was isolated by column chromatography in 14% yield (entry 1). At 160 °C the reaction afforded full conversion and piperazine was isolated by distillation in 31% yield (entry 2). Longer reaction time did not increase the yield of piperazine, though it gave rise to more by-products (entry 3). When water was used as the solvent the reaction afforded after 1 day a 70% GC-yield of piperazine and after 2 days the GC-yield of piperazine had increased to 100% (entry 4 and 5). Isolation of piperazine from water is rather difficult as the two form an azeotrope. Instead, piperazine was isolated as the hydrochloride salt in 79% yield (entry 6).

Hereby, it is shown that *N*-(2-aminoethyl)ethanolamine under the reaction conditions produces piperazine, but it does not by any means prove that the compound is an intermediate in the formation of piperazine from ethanolamine.

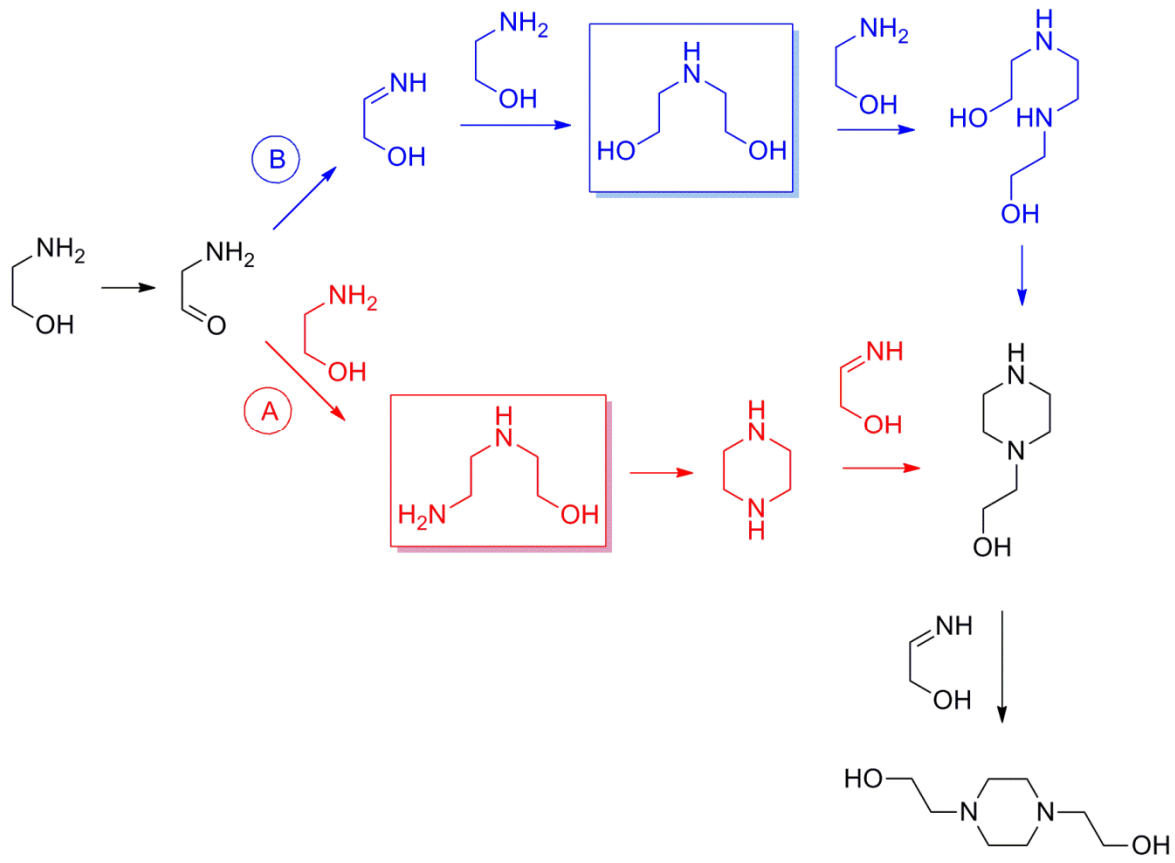
3.3.1.2 Mechanism

At some point in the reaction pathway an imine has to react with an amine. Otherwise, the observed products are not likely to be formed. From previous work on self-condensation of primary amines (chapter 2) it is known that this is possible.[151] Still, it is not known if amine oxidation is possible in the presence of an alcohol or if the imine is always formed as a result of the Voigt reaction. Williams and co-workers have reported that a ruthenium catalyzed reaction between a secondary amine and an amino alcohol afforded not only the expected amine product but also the alcohol product (scheme 55).[28] They postulate that the latter is obtained as a result of the starting amino alcohol undergoing the Voigt reaction.



Scheme 55. Proposed pathway via the Voigt reaction.

Many different suggestions to the mechanism of 1-piperazinoethanol and 1,4-piperazinediethanol formation from ethanolamine can be drafted, but none are supported by mechanistic experiments. Two possible pathways are illustrated in scheme 56.



Scheme 56. Proposed pathways for self-condensation of ethanolamine.

It is possible that reaction of ethanolamine gives piperazine via *N*-(2-aminoethyl)-ethanolamine (pathway **A**). Though, as soon as piperazine is formed it reacts with ethanolimine to give the two piperazine derivatives. The other suggestion proceeds via a diethanolamine intermediate obtained from reaction of ethanolimine with ethanolamine (pathway **B**). Unless a long polymer at some point makes a ring closing reaction with ethanolamine (or ethanolimine) it is liable to assume that 1,4-piperazinediethanol arises from reaction of 1-piperazinoethanol with ethanolamine as illustrated.

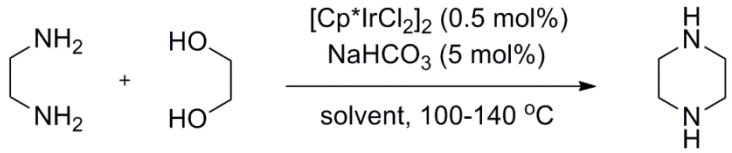
More experiments should be conducted to further elucidate the mechanism. For instance, reaction of diethanolamine with ethanolamine might reveal if pathway **B** is more likely. Reaction of 1-piperazinoethanol with ethanolamine could clarify if 1-piperazinoethanol is an intermediate in the formation of 1,4-piperazinediethanol.

1-Piperazinoethanol and 1,4-piperazinediethanol were tested for reactivity in reaction with iridium and $NaHCO_3$. In the first case, reaction in water at 140 ^{o}C and neat reaction at 160 ^{o}C afforded release of ammonia and many compounds were observed in low amounts by GC-MS analysis. However, the starting material is by far the dominating peak

in the GC-MS chromatogram, indicating a low degradation of the starting material. In the latter case, no other peaks than the starting material was observed by GC-MS analysis. Nevertheless, 20% of the starting material was consumed and this could be due to formation of higher polymers, which are not detectable by GC-MS analysis. This indicates that the two products are not completely stable under the reaction conditions. It also supports the relatively low yields of the two piperazine derivatives obtained from reaction of ethanolamine.

3.3.2 Piperazine from ethylene glycol and ethylenediamine

Piperazine derivatives have been obtained by condensation of ethylene glycol and ethylenediamine with at least one substituent on one of the starting materials. As a consequence, it was investigated if also piperazine itself could be synthesized by this strategy as illustrated in scheme 57.



Scheme 57. Piperazine from 1,2-ethylenediamine and ethyleneglycol.

Initially, screening of reaction conditions were analyzed solely by GC-MS. In all cases the only detectable product was piperazine, though isolation of this turned out to be rather tedious. Mainly toluene and water was used as solvents and these form azeotropes with piperazine. Isolation by column chromatography was unsuccessful. Instead, the product was isolated as the hydrochloride salt which in turn is a very simple work-up procedure. Unfortunately, only trace amounts of piperazine were isolated, and as a consequence an internal standard was introduced. The results are summarized in table 13.

Table 13. Reactions between 1,2-ethylenediamine and ethyleneglycol.

Entry	Solvent	Temp (°C)	Conversion (%)	products[a]
1	Toluene	110	< 10	Trace of piperazine
2	Toluene	140	100	Piperazine (15% GC)
3	Water	110	< 10	Trace of piperazine
4	Water	140	100	Piperazine (< 10%)
5	Neat	140	100	Piperazine (< 10%), (by-products)

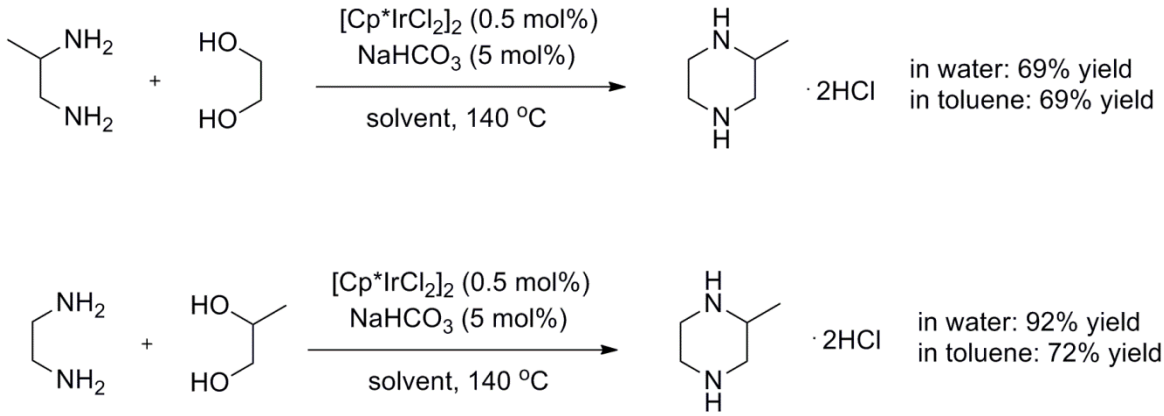
a: by GC-MS

The reaction performed in toluene at 110 °C gave poor conversion (entry 1). Increasing the temperature to 140 °C afforded full conversion, but unfortunately piperazine was only obtained in 15% yield (entry 2). Performing the reaction in water had an almost identical outcome (entry 3 and 4) and using neat reaction conditions caused formation of by-products (entry 5). With these poor results it was decided not to attempt any further optimizations of the reaction. Decomposition or formations of higher polymers are likely to have occurred.

3.3.3 Piperazines from substituted ethylene glycol and substituted ethylenediamine

3.3.3.1 2-Methylpiperazine

As the reaction of ethylene glycol and ethylenediamine failed to provide piperazine it was envisioned that introduction of a substituent might enhance reactivity due to the Thorpe-Ingold effect. This effect is described as a change in the tetrahedral angle by introduction of substituents which then enhances chemical reactivity.[152] This can be achieved by placing the substituent on either of the two starting materials as illustrated in scheme 58. In both cases the reactions proceeded smoothly and the products were isolated as hydrochloride salts in high yields.

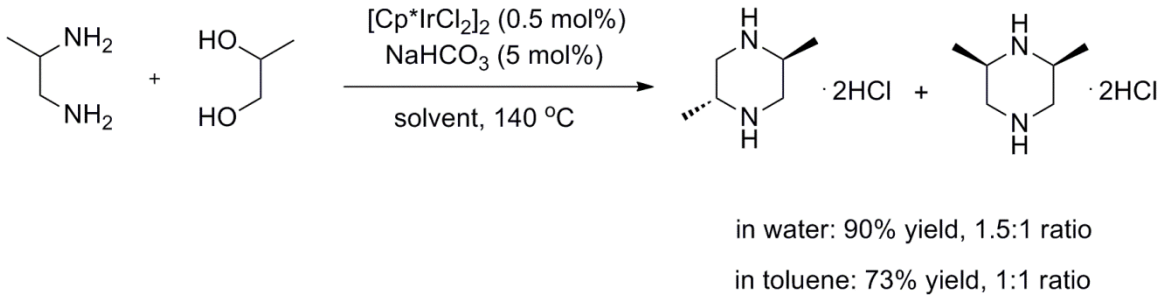


Scheme 58. Synthesis of 2-methylpiperazine.

3.3.3.2 2,5- and 2,6-dimethylpiperazine

The regioselectivity of the reaction was then investigated. The most simple starting materials, 1,2-diaminopropane and 1,2-propanediol, were used and the reaction provided

the two possible products in combined high yields (scheme 59). Unfortunately, the regioselectivity of the reaction was poor.

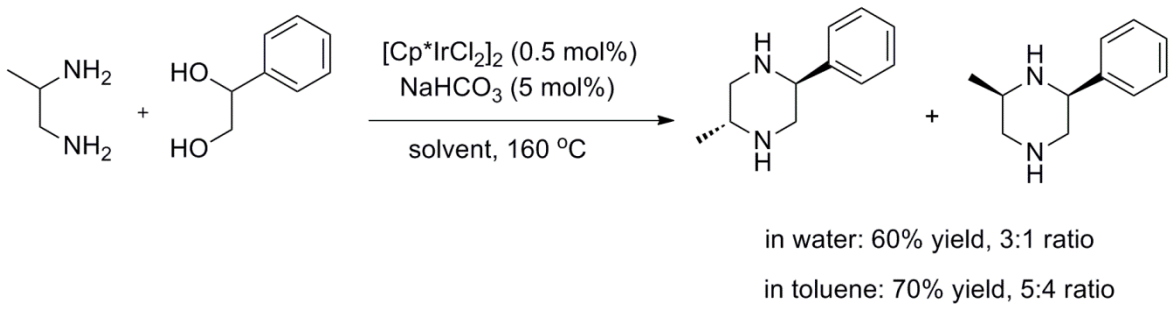


Scheme 59. Synthesis of 2,5- and 2,6-dimethylpiperazine.

The products were also isolated as the free amines, though this afforded lower yields. Isolation by flash column chromatography of products obtained from the reaction in water resulted in 34% yield in a 1:4 ratio with 2,5-dimethylpiperazine as the major product. When the reaction was performed in toluene the yield was comparable (31% yield), though in a 1:1,5 ratio with 2,5-dimethylpiperazine being the major product. Not only are the yields low, but also the regioselectivity is varying. This indicates that the products are difficult to isolate by column chromatography (despite use of TEA as co-eluent). When products were purified by distillation the yield increased to 60% (reaction performed in toluene) with a 1:1 ratio. However, in this case the toluene phase also contained products which might indicate azeotrope formation. Resultantly, isolation as the hydrochloride salts is the preferred method.

3.3.3.3 2-Methyl-5-phenylpiperazine and 2-methyl-6-phenylpiperazine

The formation of disubstituted piperazines with two different substituents was investigated with 1,2-diaminopropane and 1-phenyl-1,2-ethanediol (scheme 60). The reaction temperature had to be increased to 160 °C to afford full conversion and the products were isolated by vacuum distillation in good yields. The selectivity is dependent on the choice of solvent, with water providing a ratio of 3:1 (2,5-substitution pattern as major), whereas in toluene an almost equimolar ratio was obtained.

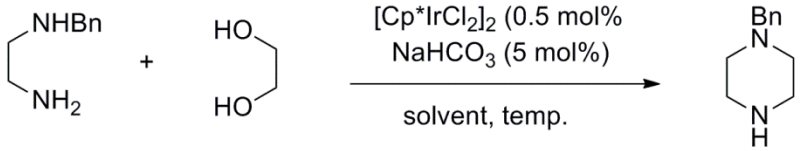


Scheme 60. Synthesis of 2-methyl-5-phenylpiperazine and 2-methyl-6-phenylpiperazine.

3.3.4 N-Benzylpiperazine

Preparation of N-monosubstituted piperazine was examined with N-benzylethylenediamine in the reaction with ethylene glycol. The results are summarized in table 14.

Table 14. Synthesis of N-benzylpiperazine.

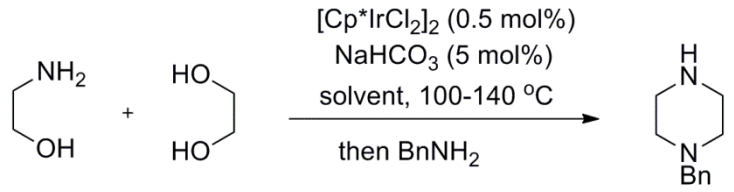


Entry	Solvent	Temp. (°C)	Conversion (%)	Yield (%)[a]
1	Water	110-140	< 10	-
2	Toluene	110-140	< 10	-
3	Toluene	160	100	63
4	None	160	100	35
5	Mesitylene	180	100	71[b]

a: after flash column chromatography. b: GC-yield.

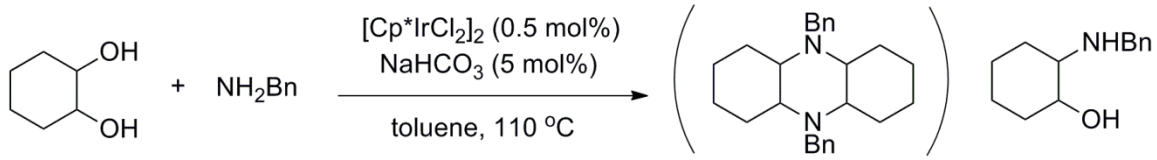
The reactions in water and toluene afforded only minor conversion of the starting material at 140 °C (entry 1 and 2). Toluene as the solvent and a reaction temperature of 160 °C proved to be the optimal conditions for full conversion and the product was isolated in 63% yield (entry 3). Interestingly, neat reaction conditions afforded a significantly lower yield of 35% (entry 5), whereas increasing the temperature only afforded a slightly higher yield (GC-yield) (entry 6).

In another attempt to make N-benzylpiperazine ethylene glycol was reacted with ethanolamine to give diethanolamine to which benzylamine was added (scheme 61). This was not successful and as described previously it is possible that ethanolamine polymerizes under the reaction conditions.



Scheme 61. Failed formation of N-benzylpiperazine from ethanolamine, ethyleneglycol and benzylamine.

As previously described N,N'-dibenzylpiperazine can be synthesized from ethylene glycol and benzylamine.[111] It was further investigated if also 1,2-cyclohexanediol could react in a similar reaction as illustrated in scheme 62.



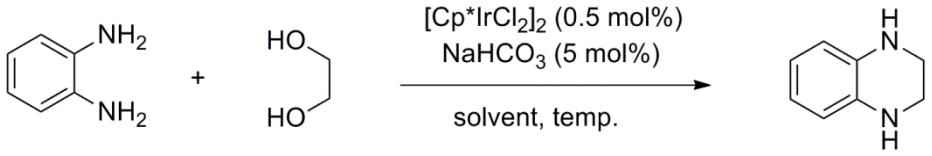
Scheme 62. Reaction between 1,2-cyclohexanediol and benzylamine.

Unfortunately, none of the desired product was observed. After reaction of one equivalent of benzylamine to give the secondary amine no further reaction was observed. The mono addition product was isolated in 54% yield and it is plausible that steric hindrance hampers further reaction with benzylamine.

3.3.5 1,2,3,4-Tetrahydroquinoxaline

So far, only aliphatic amines have been utilized for synthesis of piperazines. It was envisioned that using *o*-phenylenediamine together with ethylene glycol would produce 1,2,3,4-tetrahydroquinoxaline and the results are summarized in table 15.

Table 15. Synthesis of 1,2,3,4-tetrahydroquinoxaline.



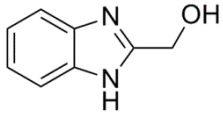
Entry	diol (eq)	Solvent	Time (h)	Temp. (°C)	Conversion of amine (%)	Yield (%)[a]
1	1	Toluene	18	110	< 10	-
2	1	Mesitylene	16	180	~ 80[b]	-
3	2	Mesitylene	18	180	100	34
4	1.5	Mesitylene	18	180	100	35

a: after flash column chromatography. b: full conversion of diol.

At 110 °C in toluene very little conversion of the starting materials was observed (entry 1). Raising the temperature to 180 °C in mesitylene resulted in full conversion of ethylene glycol, but still some 1,2-diaminobenzene remained (entry 2). Using an excess of ethylene glycol (2 eq) gave complete conversion of both starting materials and the desired product was isolated by flash column chromatography in 34% yield (entry 3). A slightly lower excess (1.5 eq) of ethylene glycol proved sufficient to maintain the same yield (entry 5).

As a result of the relatively low yield of the expected product, the choice of additive was examined. Different bases (Na_2CO_3, TEA) as well as an acidic additive (TFA) and lithium chloride were tested. A reaction in the absence of an additive was also performed. In all cases the outcome of the reaction was not improved (internal standard was used to estimate relative GC-yields) and $NaHCO_3$ remained the choice of additive. At this stage a reaction in the absence of a solvent (at 180 °C) was conducted in order to perform NMR of a crude reaction mixture. Interestingly, this revealed the presence of an unidentified product, which was later identified as 2-benzimidazolemethanol (figure 6).

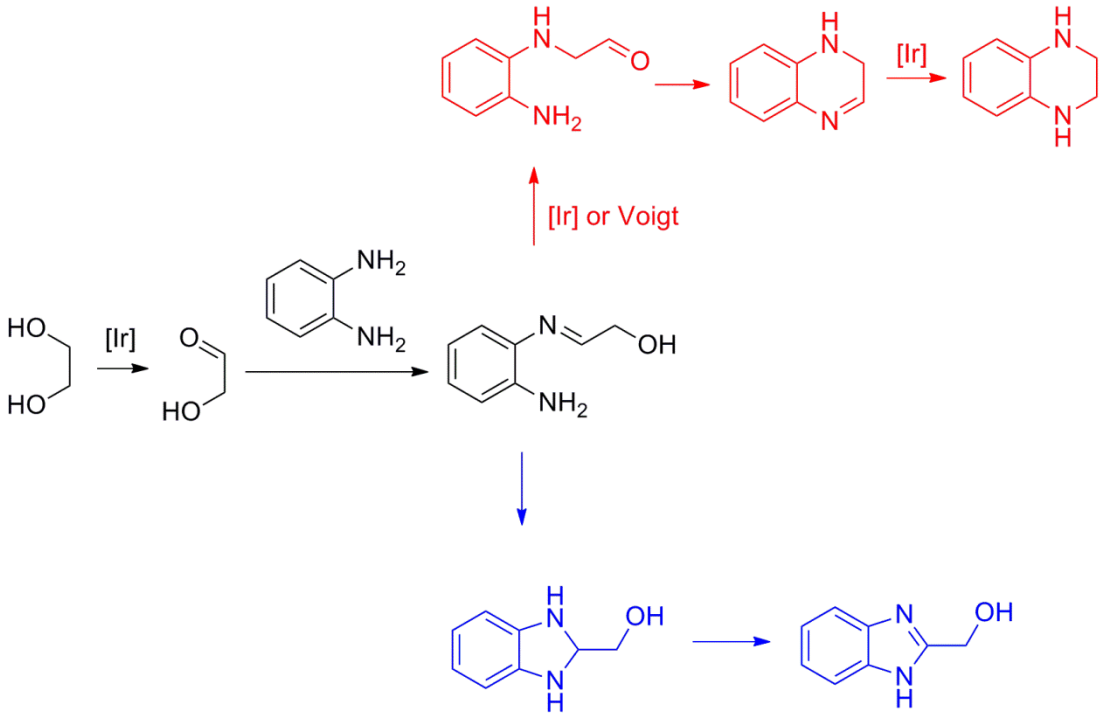
Figure 6. 2-benzimidazolemethanol.



According to NMR the ratio of this unexpected product and the desired product was 2:1. Lowering the reaction temperature or increasing the reaction time had no effect on this ratio. Isolation of 2-benzimidazolemethanol was rather tedious. Column chromatography

of the crude reaction mixture was unsuccessful as contamination with ethylene glycol was a major problem. Attempts to extract ethylene glycol into an aqueous phase were also unsuccessful as this to some extend also removed 2-benzimidazolemethanol. Fortunately, it was possible to remove the remaining ethylene glycol by vacuum distillation. Subsequent flash column chromatography in a two stage process (firstly pure EtOAc to get the desired quinoxaline-product off the column and then 10% MeOH in DCM to get the 2-benzimidazolemethanol off) separated the two products. By this work-up procedure the reaction in mesitylene at 180 ^{o}C afforded 22% of the pure product. The purification method also produced fractions of product contaminated with other by-products, and the total yield is estimated to be approximately 30%.

A plausible mechanism of the reaction is illustrated in scheme 63. After oxidation of ethylene glycol and condensation with the diamine, an α-imino alcohol is produced. This can undergo the reduction-oxidation process by iridium or the Voigt reaction to furnish the desired product. Interestingly, the α-imino alcohol can also undergo direct intramolecular attack by the amine to provide the benzimidazole product.



Scheme 63. Proposed pathways for reaction between ethyleneglycol and 1,2-diaminobenzene.

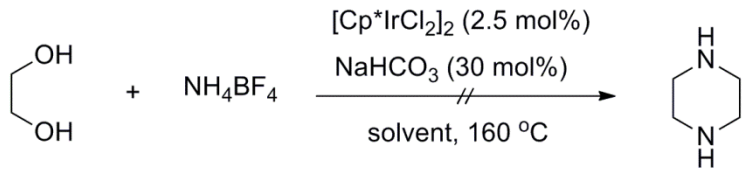
The two pathways seem to be equally favored and for that reason it was decided not to attempt any further optimization studies. Introduction of a protection group on one of

the alcohols of ethylene glycol should enhance the yield of the benzimidazole product. The expected α-imino alcohol will not be able to isomerize; hence no tetrahydroquinoxaline can be formed. If successful this could be an interesting reaction as the benzimidazole scaffold has also been identified as a privileged structure.[14] The methanol substituent can act in further functionalization of the product.

Others have performed very similar reactions. *o*-Phenylenediamine and a primary alcohol were reacted to produce benzimidazoles in presence of a ruthenium catalyst (as previously described).[61, 62] In one case $[Cp^*IrCl_2]_2$ was tested but it failed, which may be due to the fact that a basic additive was not added.[62]

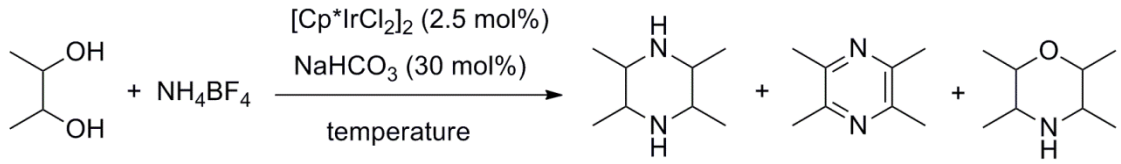
3.3.6 Piperazines from reaction of ammonium ions with diols

It has recently been reported that secondary (and tertiary) amines can be synthesized from alcohols and ammonium salts (ammonium tetrafluoroborate favors formation of secondary amines) in presence of $[Cp^*IrCl_2]_2$ and $NaHCO_3$.[75,76] Based on these results, it was envisioned that piperazines could be synthesized from the reaction between a diol and ammonium tetrafluoroborate (scheme 64).



Scheme 64. Failed synthesis of piperazine from ammonium salt and ethylene glycol.

The main challenge was isolation of the product. As the reaction is performed with an ammonium salt, the product is also a salt. Therefore aqueous acidic work-up is necessary to furnish the free amine. As already mentioned this is rather difficult as piperazine and water forms an azeotrope. Instead, a reaction was performed under neat reaction conditions and NMR of the crude reaction mixture revealed only starting material (no quantification). It was believed that maybe the Thorpe-Ingold effect would be beneficial in this case, as it also was for the condensation of diamines and diols. 2,3-Butanediol was reacted with ammonium tetrafluoroborate under neat reaction conditions (scheme 65). The outcome was analyzed by GC-MS, which revealed presence of three products; the desired piperazine together with pyrazine and morpholine derivatives.

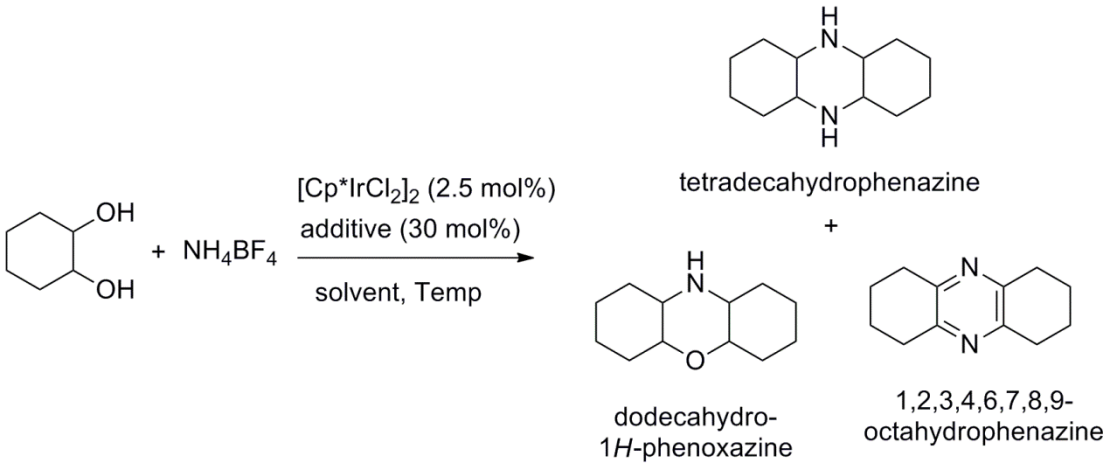


Scheme 65. Reaction between 2,3-butanediol and ammonium tetrafluoroborate.

Unfortunately, the products could not be isolated by column chromatography, and as a consequence the product distribution could not be determined. However, it was clear that it was dependant on the reaction temperature. According to GC-MS analyses the piperazine and morpholine derivatives were major products at 160 °C, whereas at 180 °C the pyrazine derivative was the major product.

Instead, 1,2-cyclohexanediol was utilized as the starting material for the reaction, as the expected products should be easier to handle during work-up. The results are summarized in table 16.

Table 16. Reactions between 1,2-cyclohexanediol and ammonium tetrafluoroborate.



Entry	diol (eq)	Solvent	additive	Temp. (°C)	Yield of dodecahydro-1*H*-phenoxazine (%)
1	1	-	$NaHCO_3$	160	-
2	1	-	$NaHCO_3$	180	-
3	2	-	$NaHCO_3$	180	30
4	1[a]	-	TFA	180	~ 45
5	2	Mesitylene	$NaHCO_3$	180	~ 45
6	2	Mesitylene	TFA	180	63
7	2	Mesitylene	-	180	50

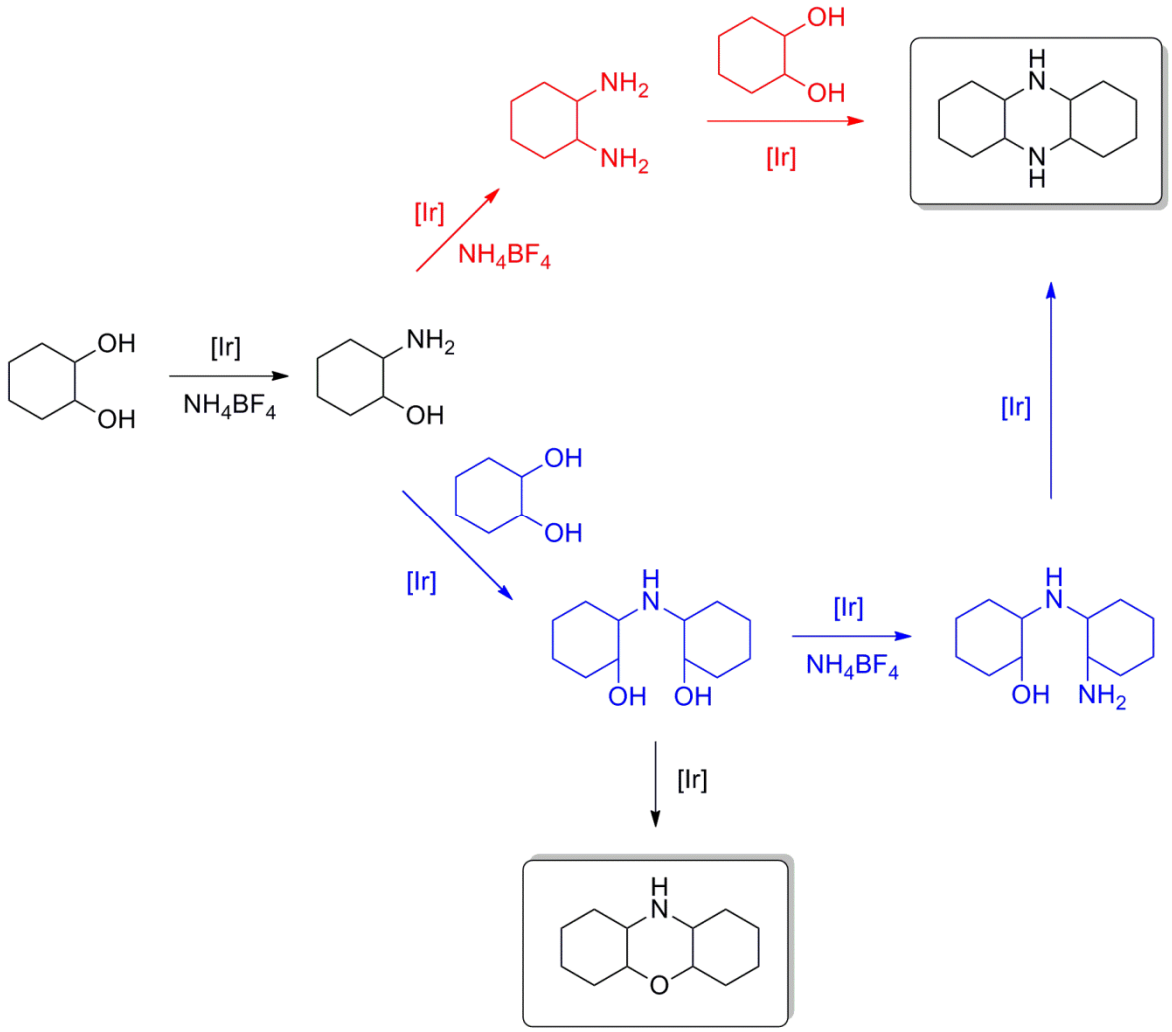
a: same product distribution when 2 eq diol is used (no isolated yield).

A reaction temperature of 160 ^{o}C was optimal to obtain full conversion of the starting materials, and it was established that this required a small excess (1.2 eq) of the diol. In this case not only the desired tetradecahydrophenazine was formed but also dodecahydro-1*H*-phenoxazine together with 1,2,3,4,6,7,8,9-octahydrophenazine was produced (entry 1). When the temperature was increased to 180 ^{o}C formation of the phenoxazine adduct became more dominating (entry 2). Focus was therefore moved to optimization of the reaction to furnish solely dodecahydro-1*H*-phenoxazine. Increasing the excess of the diol to two equivalents afforded the phenoxazine derivative as the major product (according to GC-MS analysis) (entry 3). Purification was more difficult than expected and contamination with the other products could not be avoided. Merely 30% yield of phenoxazine product was obtained. Further examination of reaction conditions revealed that an acidid additive was a better compared to base (entry 4). Using catalytic amounts of TFA increased the formation of the phenoxazine derivative and suppressed the formation of the piperazine derivative. Additionally, with use of TFA as additive the product ratio did not change when equivalent amounts of diol were used in the reaction. An isolated yield of 45% of the phenoxazine product was obtained when equimolar amounts of the starting materials were reacted in the presence of the catalyst and TFA. Reactions performed in a solvent (mesitylene) were examined using two equivalents of the diol. The use of acid or base as additive afforded in both cases almost solely the phenoxazine derivative according to GC-MS analysis. A reaction performed with $NaHCO_3$ as the additive afforded approximately 45% yield (entry 5), whereas with TFA the yield increased to 63% (entry 6). A reaction in the absence of an additive lowered the yield to approximately 50% (entry 7). Reactions performed at a higher reaction temperature of 200 ^{o}C for either 6 or 28 hours did not have any effect on the product distribution (determined by GC-MS analysis – no quantification).

In conclusion, the best results were obtained by reaction of the ammonium salt and the diol in a 1:2 ratio in mesitylene at 180 ^{o}C and with catalytic amounts of TFA and $[Cp^*IrCl_2]_2$. This afforded a yield of 63% of the phenoxazine product.

The suggested reaction pathways are illustrated in scheme 66. Initially, the diol is oxidized and reacted with the ammonium salt to generate 2-aminocyclohexanol. After oxidation of the alcohol it can either react with the ammonium salt or with 1,2-cyclohexanediol. In the first case, the reaction gives 1,2-diaminocyclohexane, which then reacts with 1,2-cyclohexanediol to give the desired product. In the latter case, the reaction with 1,2-cyclohexanediol furnishes a diol intermediate that upon reaction with the ammonium salt

affords an amino alcohol intermediate. This amino alcohol is setup for an intramolecular reaction to afford the desired product. Interestingly, the diol intermediate can undergo an intramolecular reaction between the two alcohols to furnish the morpholine derivative instead of reacting with the ammonium salt. This pathway is apparently more favored.

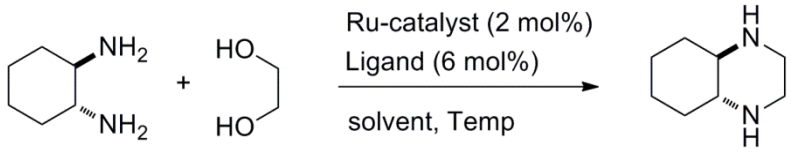


Scheme 66. Proposed pathways for reaction between 1,2-cyclohexanediol and ammonium salt.

3.3.7 Piperazines by ruthenium catalysis

As it has already been explained, ruthenium catalysts are generally cheaper than iridium catalysts and in the literature there have been many examples of ruthenium catalyzed condensations of primary amines and alcohols (see chapter 1 and 2). Consequently, it was envisioned that the reaction between 1,2-diols and 1,2-diamines in the presence of a ruthenium catalyst would furnish piperazines. The results are summarized in table 17.

Table 17. Ruthenium catalyzed reactions of 1,2-diaminocyclohexane and ethyleneglycol.

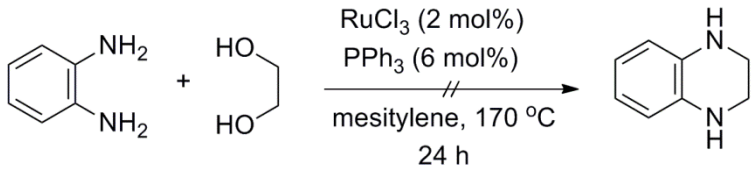


Entry	Catalyst	Ligand	Solvent	Additive	Temp. (°C)
1	$RuCl_3$	PPh_3	Water	-	100
2	$RuCl_3$	PPh_3	Toluene	-	110
3	$RuCl_3$	PPh_3	-	-	110
4	$RuCl_3$	PPh_3	Toluene	-	140
5	$RuCl_3$	PPh_3	Mesitylene	-	180
6	$RuCl_3$	PPh_3	-	-	180
7	$RuCl_3$	PPh_3	Mesitylene	-	(110)170
8	$RuCl_3$	PPh_3	-	-	(110)170
9	$RuCl_3$	PPh_3	Mesitylene	$NaHCO_3$	(110)170
10	$RuCl_3$	PPh_3	-	$NaHCO_3$	(110)170
11	$RuCl_3$	PPh_3	Mesitylene	TFA	(110)170
12	$RuCl_3$	PPh_3	-	Stoic. TFA	(110)170
13	$RuCl_3$	Xantphos	Mesitylene	-	(110)170
14	$RuCl_3$	Xantphos	Mesitylene	-	(110)170
15	$RuCl_2(PPh_3)_3$	-	Mesitylene	-	(110)170
16	$RuCl_3$ (x5)	PPh_3	Mesitylene	-	(110)170

It was chosen to focus predominantly on $RuCl_3$ and triphenylphosphine as the catalytic system, as it is cheap and it has been widely used in reactions of primary/secondary amines and alcohols with good results (see chapter 1 and 2). Initially, the optimal reaction temperature was examined. Unfortunately, no product was observed when performing the reaction at temperatures ranging from 100-180 °C in different solvents as well as under neat reaction conditions (entry 1-6). Former PhD student Matyas Tursky had used the same catalyst and ligand for indole synthesis (more details in chapter 4). He found that more reliable (and reproducible) results were obtained when the reaction mixture was initially heated to 110 °C for 1 hour (optimal temperature for generation of the active catalyst) and then increased to 180 °C for the remainder of the reaction time. Unfortunately, this had no effect in this project (entry 7+8). Neither did addition of a catalytic amount of base ($NaHCO_3$) or acid (TFA) change the outcome of the reaction (entry 9-11). It was then tested if a fully protonated amino group would increase

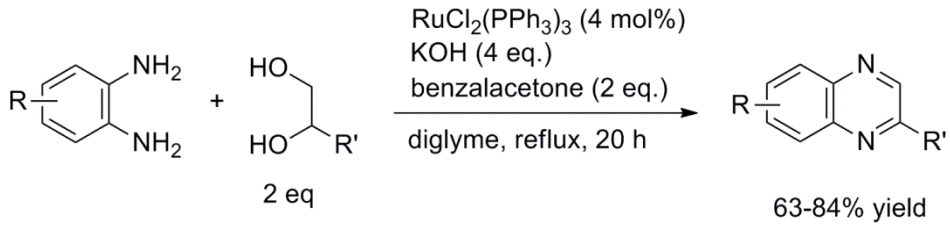
reactivity of the starting material, and a stoichiometric amount of TFA was therefore used in the reaction. However, the reaction did not afford the desired product (entry 12). Focus was then moved to the catalytic system. Instead of the monodentate ligand triphenylphosphine, a bidentate ligand (xantphos) was tested for reactivity, but with no success (entry 13-14). Also, the preformed active catalyst $RuCl_2(PPh_3)_3$ failed completely (entry 15). Finally, it was examined if an increased catalyst loading would increase reactivity and a reaction with 10 mol% ruthenium was performed, but still product formation did not occur (entry 16).

It is possible that the diamine starting material coordinates to ruthenium and hereby deactivates the catalyst. Therefore, *o*-phenylenediamine was used instead of 1,2-diaminocyclohexane (scheme 67). Unfortunately, reaction between the two starting materials did not take place.



Scheme 67. Failed attempt to synthesise 1,2,3,4-tetrahydroquinoxaline.

It should be noted that Cho and Oh in 2006 published a method for preparation of the fully aromatized quinoxaline as illustrated in scheme 68.[71] Excess amount of diol increased the yield from 50% to 75%. More importantly, addition of KOH was crucial as a reaction in the absence of this afforded only 18% of the product. Unfortunately, they did not examine if a catalytic amount of KOH is sufficient.



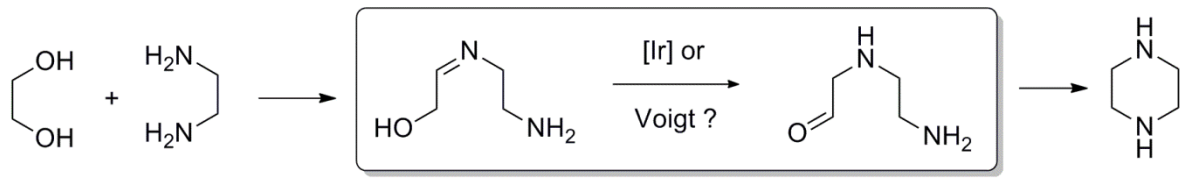
Scheme 68. Literature example on the use of aromatic diamines.

An excess amount of base may be required for the reaction described herein and this could explain the poor results rather than the catalyst inactivation by coordination to the

diamine. Consequently, it was decided not to attempt any further optimizations of the reaction.

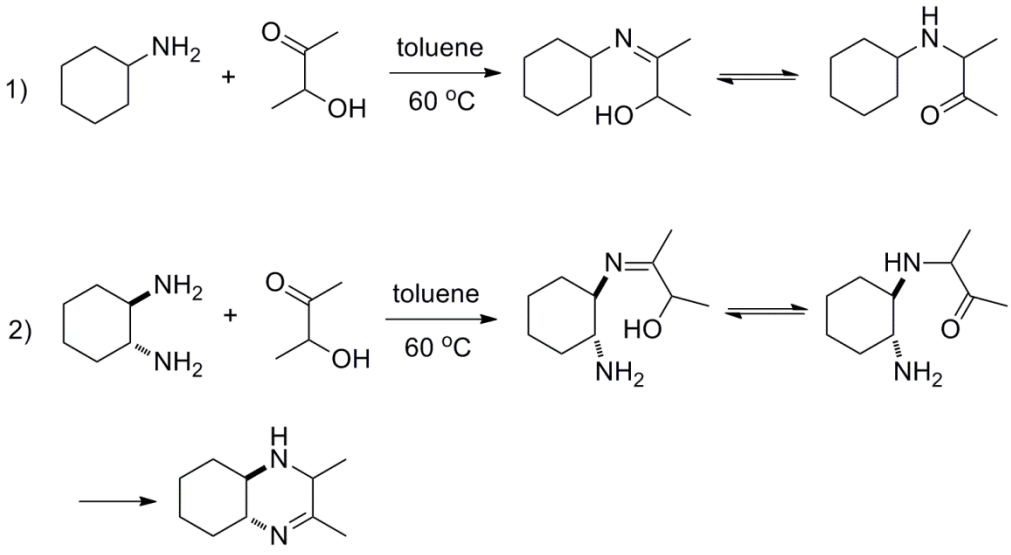
3.3.8 Mechanism of the piperazine reaction

Formation of piperazines from diols and diamines is believed to start with oxidation of the alcohol to the carbonyl compound followed by condensation with the amine to furnish an imine. This is supported by both mechanistic and computational experiments (formation of secondary amines from primary alcohols and amines) as described in details in chapter 1. However, when diamines and diols are condensated, the corresponding imine is actually an α-imino alcohol. It has not been established how this further reacts. Obviously, it can undergo the reduction/oxidation process catalyzed by iridium to provide an α-amino ketone, but this can also be achieved in a Voigt reaction (scheme 69).



Scheme 69. Mechanism via iridium reduction/oxidation sequence or via the Voigt reaction?

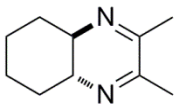
In the pursuit to elucidate if the Voigt reaction plays a significant role in the reaction mechanism the reactions illustrated in scheme 70 were performed.



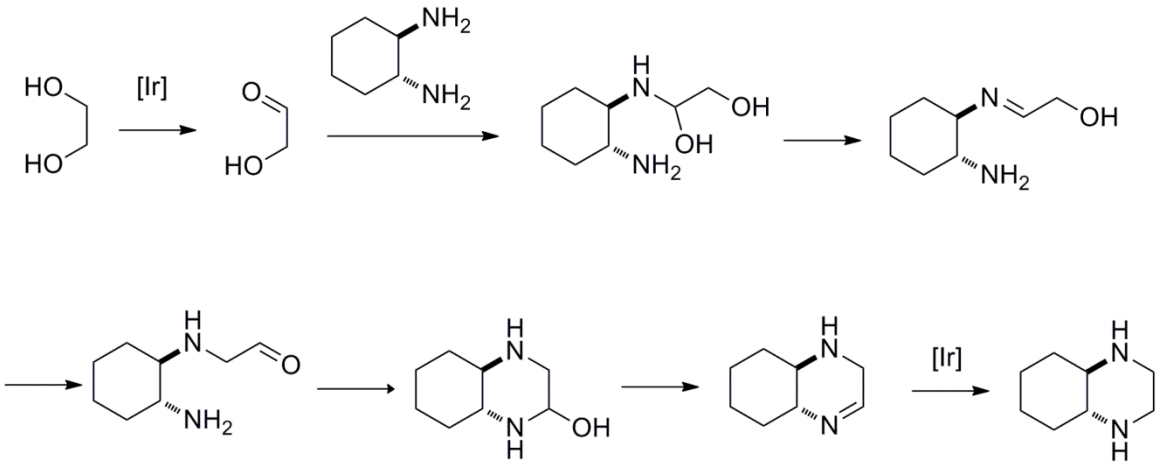
Scheme 70. Mechanistic experiments.

The reaction of cyclohexylamine and acetoin in toluene at 60 ^{o}C (reaction 1) revealed that the α-aminoketone was the sole product after 1 hour. As the temperature in the piperazine synthesis is higher (110-180 ^{o}C) and with longer reaction times (12-24 hours) the result strongly indicates that the Voigt reaction is dominating. The same reaction was performed in presence of the iridium catalyst and this did not increase the reaction rate of the transformation, which also supports that iridium has a very limited effect on the formation of the α-amino ketone. Further examination of the reaction mechanism was conducted with 1,2-diaminocyclohexane (reaction 2). However, in this case the expected cyclic imine was not observed. Instead the cyclic diimine illustrated in figure 7 was the sole product after 4 hours.

Figure 7. 2,3-dimethyl-5,6,7,8,9-hexahydroquinoxaline



To the best of our knowledge the expected cyclic imine is too unstable and is oxidized immediately to the cyclic diimine (even at inert conditions). In this way, these results also support that the Voigt reaction plays a significant role in the formation of piperazines. With these results the complete transformation is illustrated in scheme 71.



Scheme 71. Proposed pathway for synthesis of piperazines from 1,2-diols and 1,2-diamines.

Interestingly, this implies that iridium is only involved in the first and the last step of the pathway; initially oxidation to the aldehyde (or ketone) and the final reduction of the imine to the secondary amine. It must be noted that it has not been established if the intermediates are coordinated to iridium throughout the process.

3.4 Conclusion

The work described in this chapter will be combined with the published results of Nordstrøm and Madsen[111] for a full paper on piperazine synthesis from amines and alcohols and the manuscript is currently in preparation.

Ethanolamine was not suitable as a starting material for the synthesis of piperazine as it formed N-mono- and di-N-substituted ethylalcohol piperazines in almost 60% combined yield. It is assumed that ethanolamine also undergo polymerization, which could account for the moderate yields of isolated products.

It was established that the Thorpe-Ingold effect plays a significant role in the reaction as unsubstituted diols and diamines failed to produce piperazine. With just one methyl substituent (on the diol or the diamine) the corresponding product was obtained in high yields (69-92%). Substituents on both starting materials (methyl or phenyl) also furnished the products in high yields (60-90%). In general, the 2,5-disubstituted product was favored, but in some cases the 2,6-disubstituted piperazine was formed in equal amounts.

N-benzylpiperazine was obtained in 71% yield from ethylene glycol and N-benzylethylenediamine, whereas reaction of 1,2-cyclohexanediol and benzylamine failed to provide 5,10-dibenzyltetradecohydrophenazine. Instead, 2-(benzylamino)cyclohexanol was formed in 54% yield as a result of only monoaddition of benzylamine to the diol. Steric hindrance is believed to be the reason for no further reaction with benzylamine.

Use of *o*-phenylenediamine turned out to be more difficult than anticipated as there is a competing reaction to form 2-benzimidazolemethanol. The expected quinoxaline product and the benzimidazole product were formed in almost equimolar amounts in a total yield of 65%.

Ammonium salts were examined as the source of nitrogen in the reaction with diols to furnish piperazines. Substituents on the diol proved to be crucial and with 1,2-cyclohexanediol and ammonium tetrafluoroborate not only the piperazine derivative was formed, but also the corresponding morpholine derivatives. Optimized reaction conditions furnished the morpholine product in 63% yield.

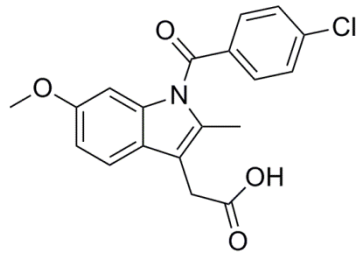
Unfortunately, piperazine synthesis from 1,2-diaminocyclohexane and ethylene glycol by ruthenium catalysis failed completely. It is plausible that the reaction requires excess amount of base as this has been reported in the literature for a similar reaction. However, it was not attempted as this would result in poor atom economy.

The mechanistic experiments revealed that the Voigt reaction plays an important role in the formation of the piperazine ring. Additionally, it also supports the results obtained from the reactions of ethanolamine.

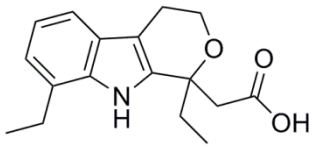
4 Indoles

Indoles are widely used in products ranging from dyes and plastics to flavor enhancers, perfumes and pharmaceuticals.[153] As already explained in chapter 1 the indole scaffold has been identified as a privileged structure. In figure 8 a few of the many indole containing pharmaceuticals are illustrated.

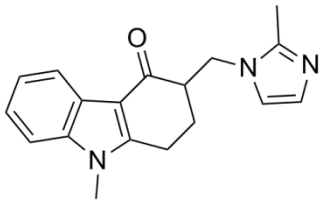
Figure 8. Indole containing pharmaceuticals.



Indomethacin by Camber Pharmaceuticals
(e.g. to reduce fever and pain)



Todolac by Norphama (for arthritis)



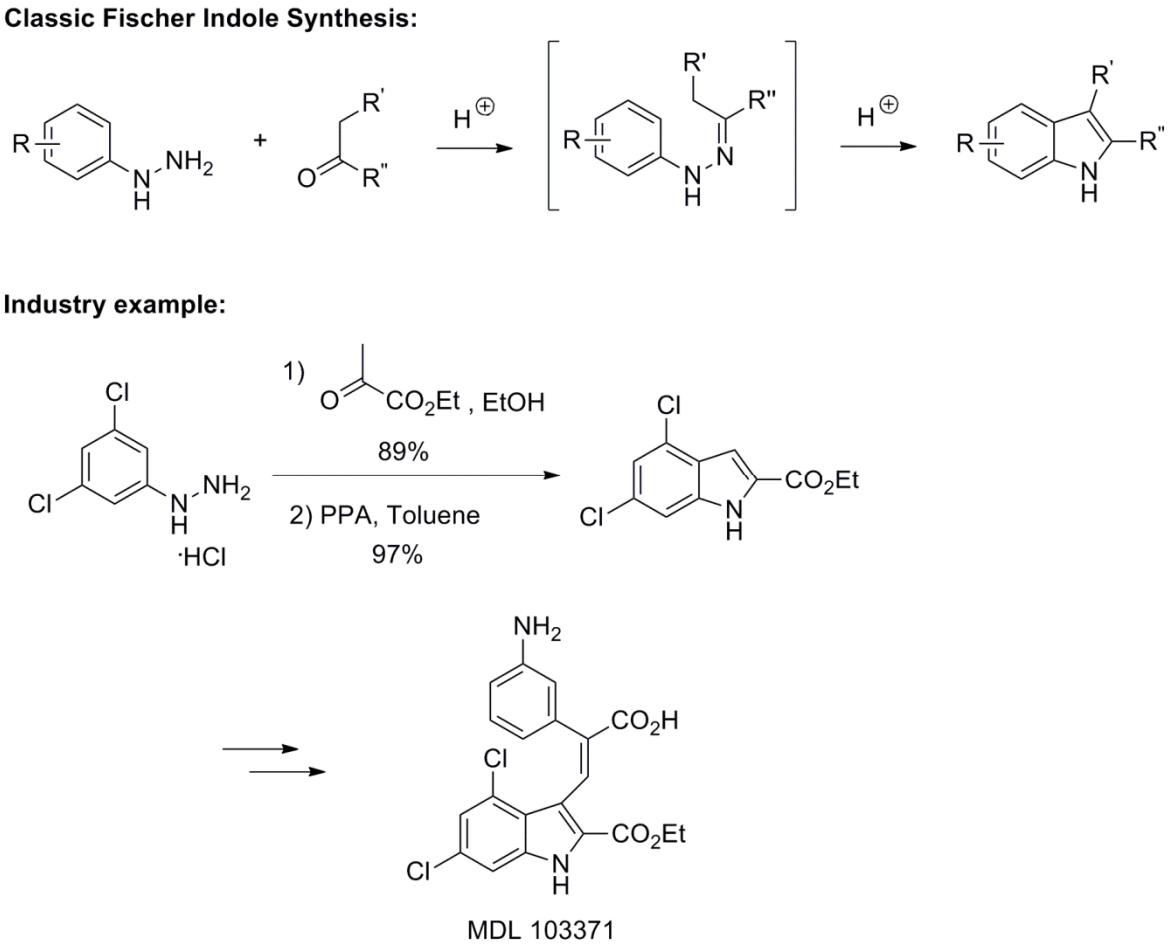
Zofran by GlaxoSmithKline
(e.g. for treatment of nausea)

4.1 Indole syntheses

Syntheses of indoles have been investigated for more than a century and due to its importance in the pharmaceutical industry many methods have been developed. Interestingly, the field still receives great attention with new publications every week. Herein, some of the classical routes to indoles as well as more recent methods will be briefly described. As a consequence of the almost immeasurable number of procedures focus will predominantly be on one-pot syntheses that tolerate a wide variety of substitution patterns. Additionally, the described methods will not include those where the benzene moiety is formed as a part of the indole synthesis.

4.1.1 From arylhydrazines or arylhydrazones

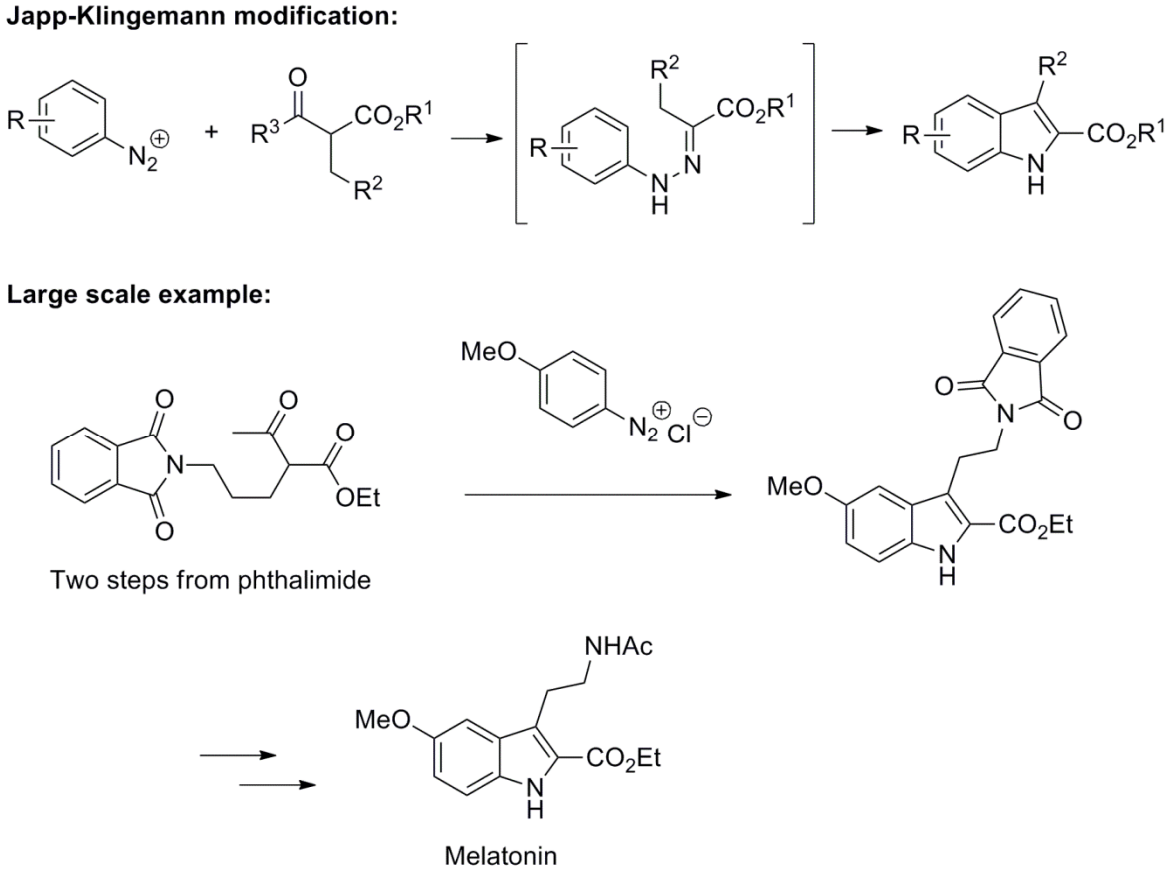
One of the most widely used methods for indole synthesis is the Fischer indole synthesis developed in 1883.[154-156] It involves reaction between an arylhydrazine and a ketone or aldehyde to furnish the indole skeleton via an arylhydrazone (scheme 72). The reaction is still undergoing constant modification and optimization[157-159]. Nevertheless, the reaction in its original form is used in many large scale syntheses and herein it is exemplified with the kilogram production of MDL 103371, a potential candidate for treatment of stroke (scheme 72).[154] The major drawback from an environmental perspective is the toxicity of the arylhydrazines.



Scheme 72. Fischer indole syntheses.

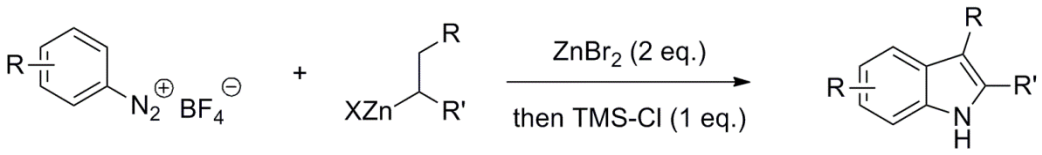
A commonly used modification of the Fischer indole synthesis is the Japp-Klingemann procedure, involving the reaction of an aryldiazonium salt with a β-keto ester (or acid) (scheme 73).[160] The formed indole always contains an ester moiety in the 3-position, which can either be further functionalized or undergo decarboxylation.[161] This method is

also used on a kilogram scale and is exemplified in scheme 73 with the synthesis of Melatonin.[162]



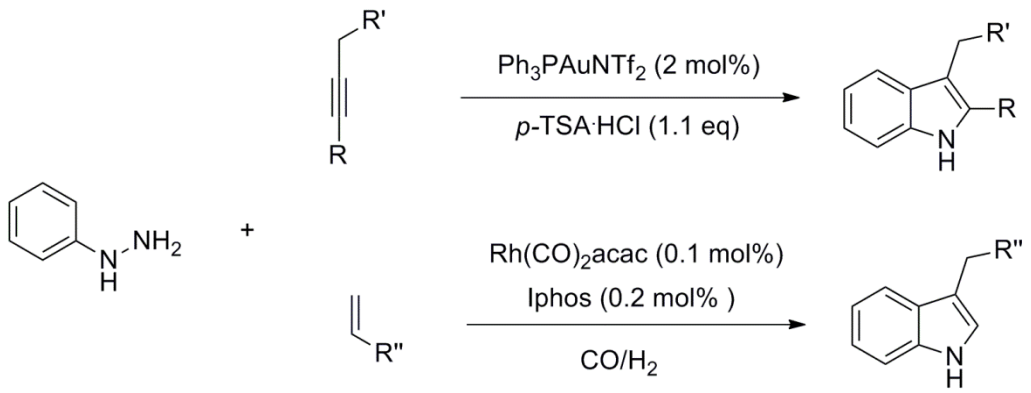
Scheme 73. Japp-Klingemann modification of the Fisher indole synthesis.

Aryldiazonium salts can also be converted into 2,3-disubstituted indoles when reacted with alkyl zinc reagents (scheme 74).[163]



Scheme 74. Use of aryldiazonium salts.

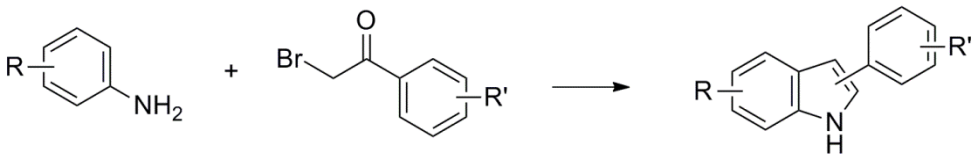
Other modifications of the Fischer indole synthesis include the reaction between an arylhydrazine and an alkyne catalyzed by gold[164] and the reaction between an arylhydrazine and a terminal alkene catalyzed by rhodium[165] (scheme 75). These methods furnish 2,3-disubsituted and 3-substituted indoles, respectively.



Scheme 75. Catalytic modifications of the Fisher indole synthesis.

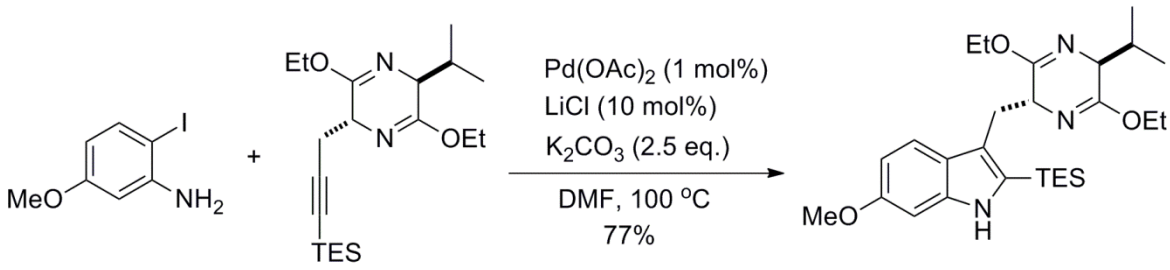
4.1.2 From anilines or *ortho*-haloaniline

Synthesis of indoles from anilines and α-haloketones is today known as the Bischler indole synthesis (scheme 76).[166,167] It was developed in the late 1900's, but has since received little attention. Nevertheless, a few improved examples have been reported, e.g. use of microwave irridation[168] and addition of additives[169]. In the original Bischler indole synthesis the substituent always ends up in the 2-position, though with the more recent optimization also selectivity towards the 3-position has been reported (e.g. when additives are used).[169] Despite the limited use of this procedure the reaction is still interesting from a mechanistic point of view (more details in section 4.3.3).



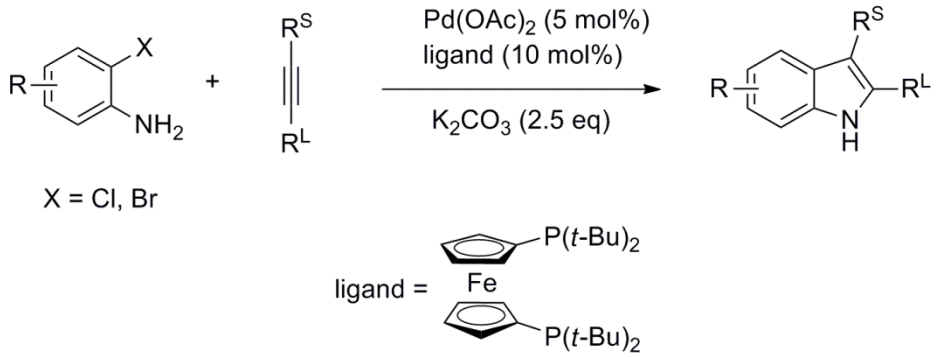
Scheme 76. Bischler indole synthesis.

The Larock indole synthesis from 1991 involves reaction between an *ortho*-iodoaniline and an alkyne catalyzed by palladium.[170] This procedure is widely used on a large scale[154,156] and an example is illustrated in scheme 77.[171]



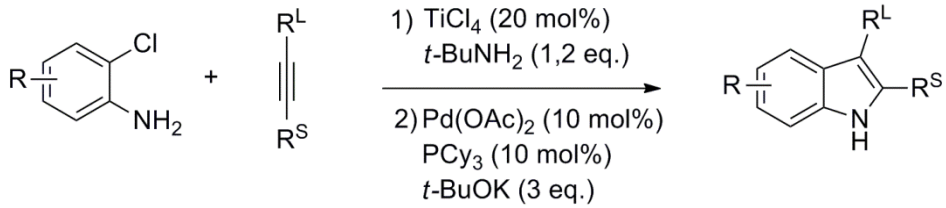
Scheme 77. Larock indole synthesis.

Senanayake and co-workers have optimized the Larock indole synthesis, which makes it possible to use the much cheaper and more readily available chloro- and bromoanilines (scheme 78).[172]



Scheme 78. Optimized Larock indole synthesis.

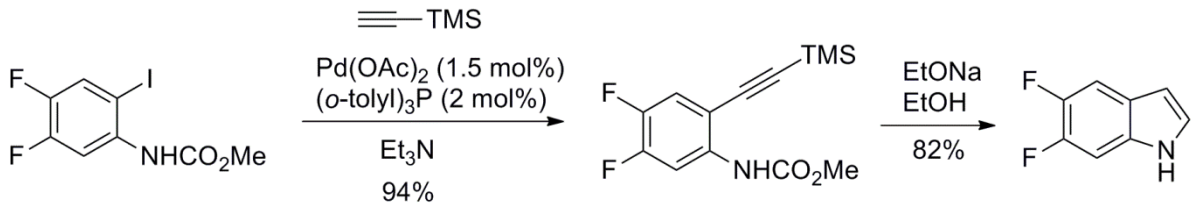
Utilization of a titanium catalyst in the Larock indole synthesis (scheme 79) changes the selectivity of the reaction and the smallest substituent ends up in the 2-position and the largest subsitutent in the 3-position.[173]



Scheme 79. Reversing the selectivity of the Larock indole synthesis.

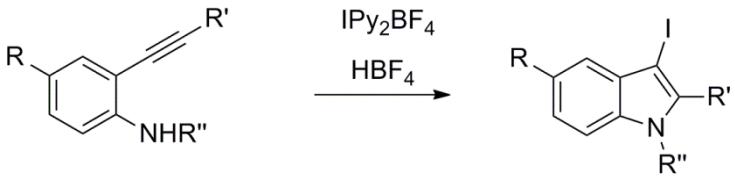
Ortho-haloanilines react with terminal alkynes to give 2-substituted indoles in a two-step process; firstly, a Sonogashira coupling catalyzed by palladium or copper, which is followed by a cyclization reaction often mediated by base. Many examples of this type of

reaction have been reported[154,156], and herein it is exemplified with the multi-kilogram synthesis of the indole shown in scheme 80.[174]



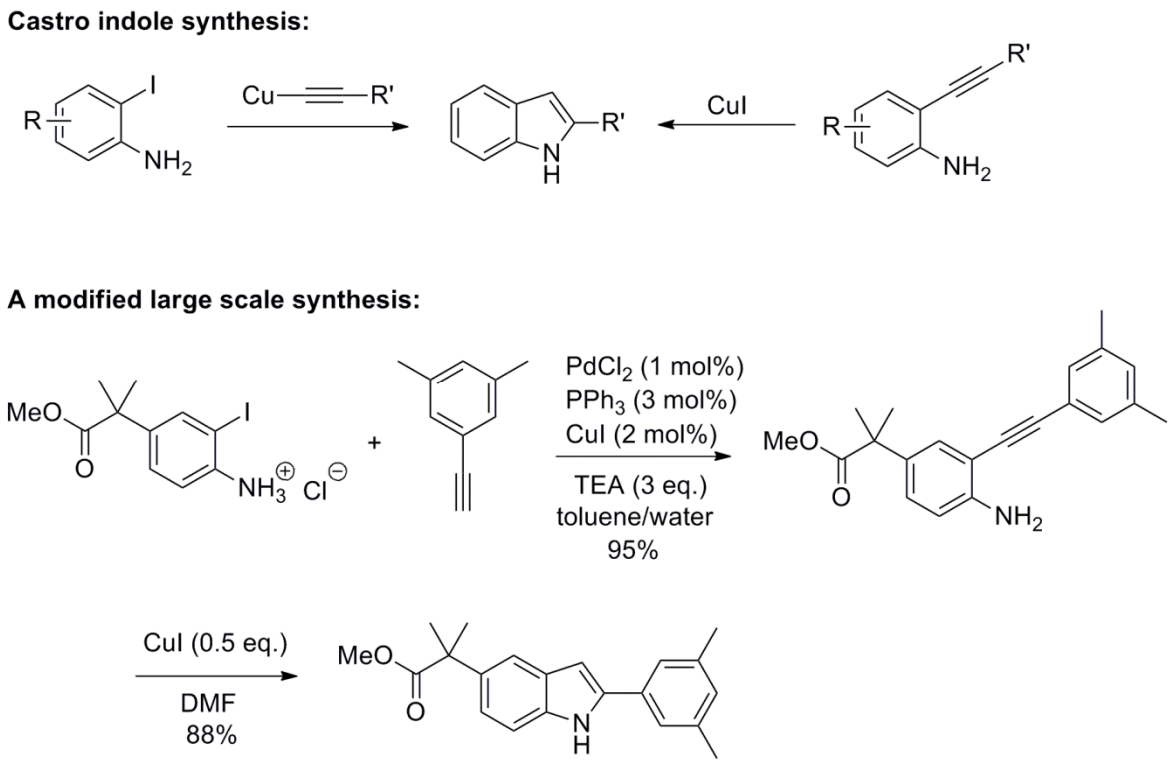
Scheme 80. Indoles from *ortho*-haloanilines and terminal alkynes via a Sonogashira coupling.

If a Sonogashira coupling product is reacted with IPy_2BF_4 this will lead to incorporation of an iodine in the 3-position.[175] This can serve as a handle for further functionalization of the compound (scheme 81).



Scheme 81. Use of a Sonogashira coupling product for indole formation.

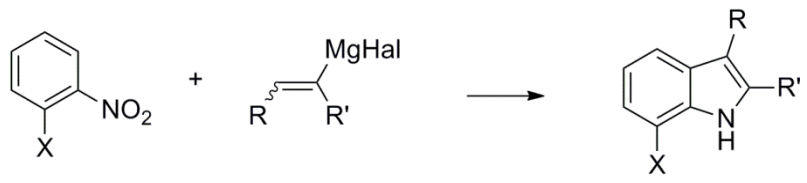
The Castro indole synthesis was developed in the 1960's and was the first reported use of copper for preparation of indoles.[176] It involves reaction of either iodoanilines with cuprous acetylides or *ortho*-alkynylanilines with copper(I) salts (scheme 82).[177-179] The reaction is also exemplified in scheme 82 as a large scale synthesis with slightly modified reaction conditions.[180] The major disadvantages are the use of excess acidic additive and a relatively high use of copper iodide for the ring closing reaction.



Scheme 82. Castro indole syntheses.

4.1.3 From nitroarenes

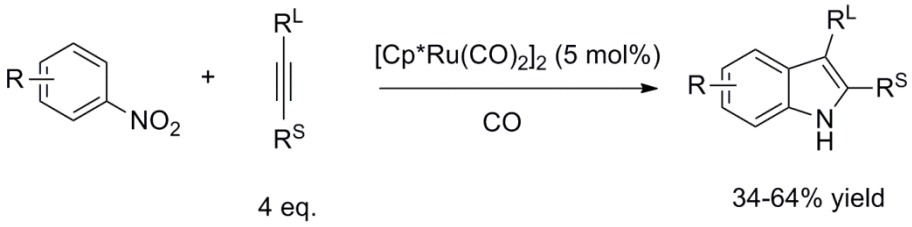
Use of nitroarenes and vinylmagnesium bromide for the synthesis of 7-substituted indoles is best known as the Bartoli indole synthesis from 1989.[181] It is crucial for the reaction that the nitroarene has a substituent in the *ortho*-position. Later, it was found that the reaction also tolerates substituents on vinylmagnesium bromide furnishing 2,3,7-substituted indoles (scheme 83).[182]



Scheme 83. Bartoli indole synthesis.

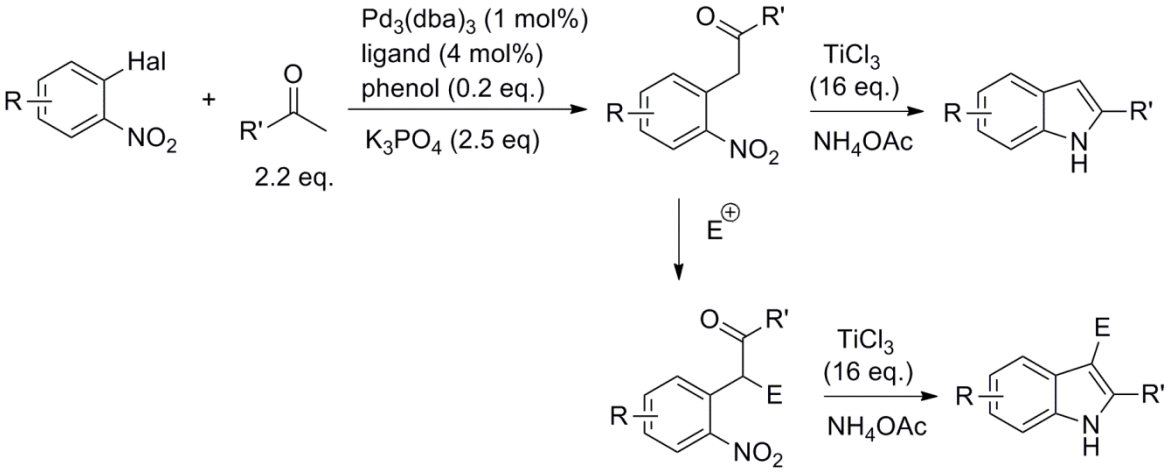
Several modifications of the procedure have been published avoiding the requirement of a substituent in the *ortho*-position of the nitroarene. One example is the Dobbs modification, which involves use of *ortho*-bromonitrobenzenes in reaction with the vinylmagnesium halides.[183] This affords an indole with a bromide in the 7-position (as well

as substituents in the 2- and 3-position) and is removed in a radical reaction with AIBN and Bu_3SnH. Another modification is reaction of nitroarenes with alkynes in the presence of a ruthenium catalyst as illustrated in scheme 84.[184]



Scheme 84. Modified Bartoli synthesis.

Buchwald and co-workers have used *ortho*-halonitrobenzenes in the reaction with ketones to give the corresponding *ortho*-nirobenzenyl ketone (scheme 85).[185] Without purification this is treated with a great excess of $TiCl_3$ to provide the 2-subsituted indole. The *ortho*-nitrobenzenyl ketone can also be reacted with an electrophile to provide an additional substitution in the 3-position. A major disadvantage, besides the poor atom economy due to at 16-fold excess of titanium(III) chloride, is the toxicity of nitroarenes.

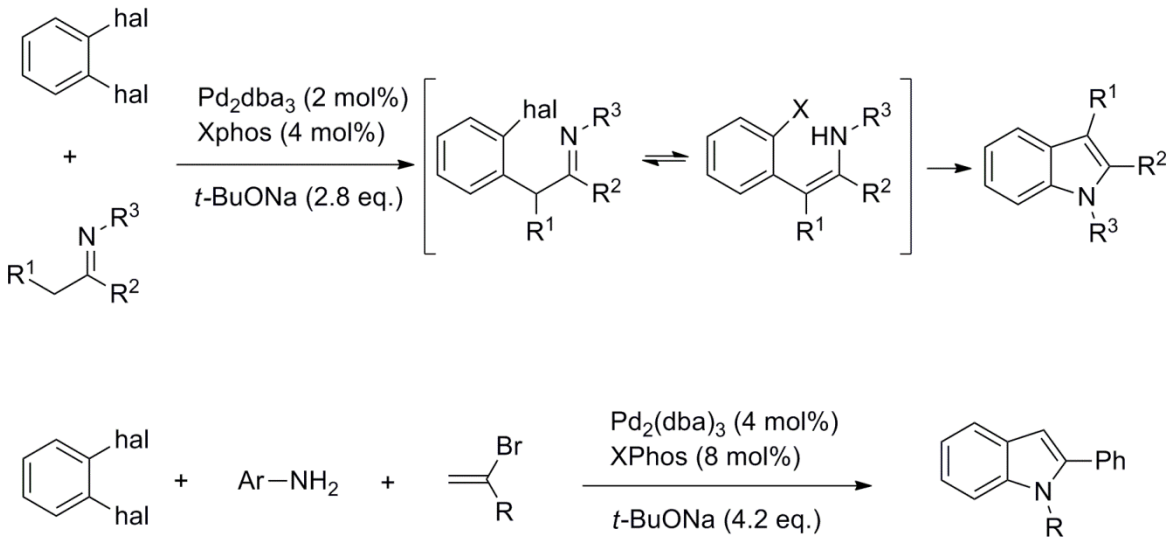


Scheme 85. Reaction between *ortho*-halonitrobenzenes and ketones.

4.1.4 From *ortho*-dihaloarenes

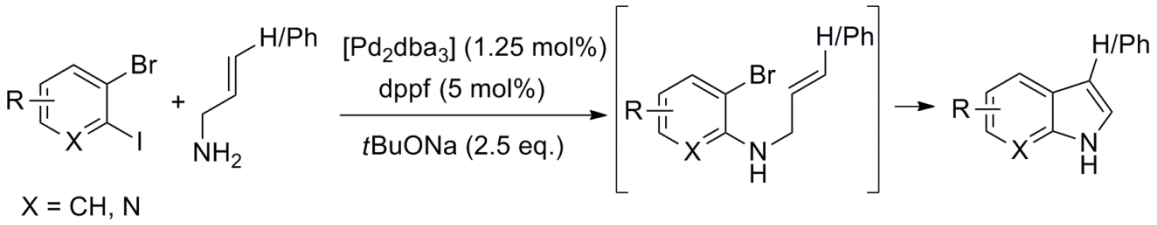
Indoles can also be achieved from *ortho*-dihaloareanes in the reaction with imines in the presence of a palladium catalyst (scheme 86).[186,187] The intermediate undergoes a Buchwald-Hartwig amination to furnish the indole, which is either 2,3- or 2-substituted depending on the nature of the iminie. As also illustrated in scheme 86 the imine can be

generated *in situ* and therefore the indole is obtained in a one-pot synthesis from an *ortho*-dihaloarene, an arylamine and a terminal alkene.[186]



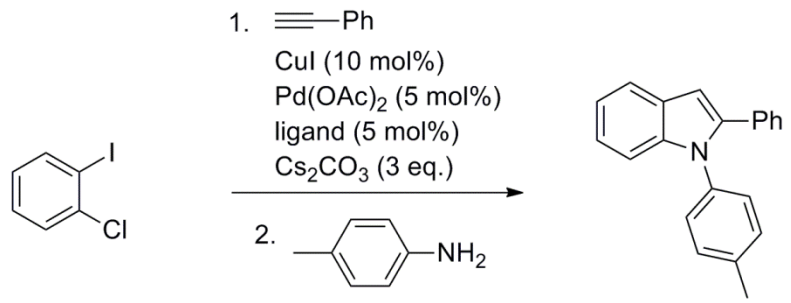
Scheme 86. Synthesis of indoles from *ortho*-dihaloarenes.

Ortho-dihaloarenes reacts with internal alkenes in the presence of a palladium catalyst (scheme 87).[188] The first step is N-arylation, which is followed by an intramolecular Heck-coupling to furnish the indole skeleton.



Scheme 87. Use of internal alkenes in reaction with *ortho*-dihaloarenes.

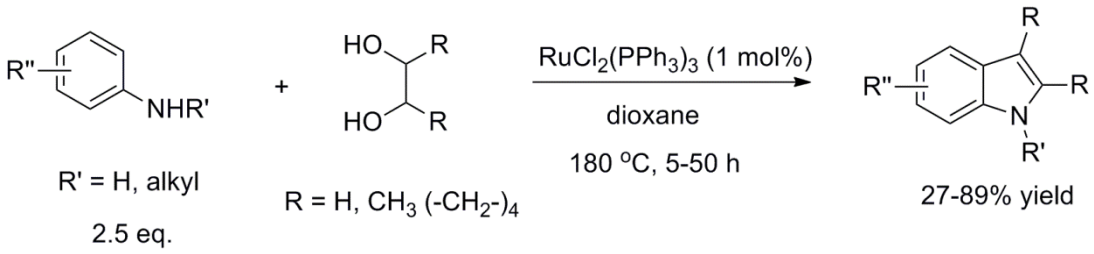
Ackerman investigated the Sonogashira coupling of terminal alkynes with *ortho*-dihaloarenes and found a catalytic system that provides the indoles in a one-pot procedure as illustrated in scheme 88.[189] The reaction was further optimized in a manner that tolerate more sterically hindered N-substituents[190] and also utilization of a much cheaper nickel catalytic system has been reported.[191]



Scheme 88. Reaction between *ortho*-dihaloarenes and terminal alkynes.

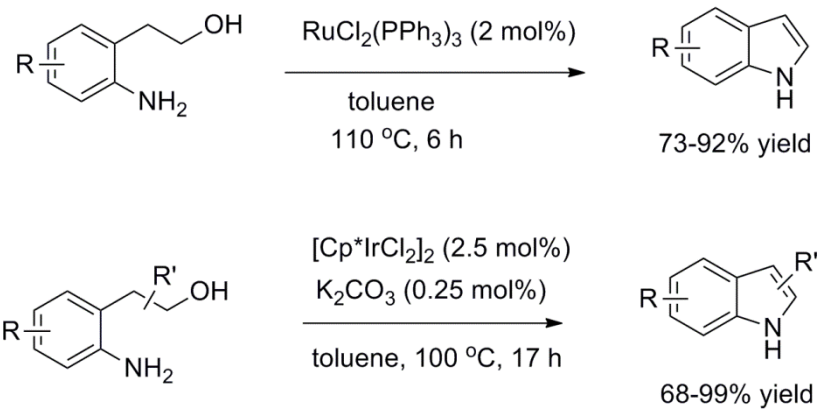
4.1.5 By N-alkylation of amines with alcohols by Ru- or Ir-catalysis

One of the first reports concerning synthesis of indoles by the hydrogen transfer methodology was published in 1986 by Watanabe and co-workers.[65] Anilines were reacted with diols in the presence of a ruthenium catalyst affording the indoles in moderate to high yields (scheme 89).



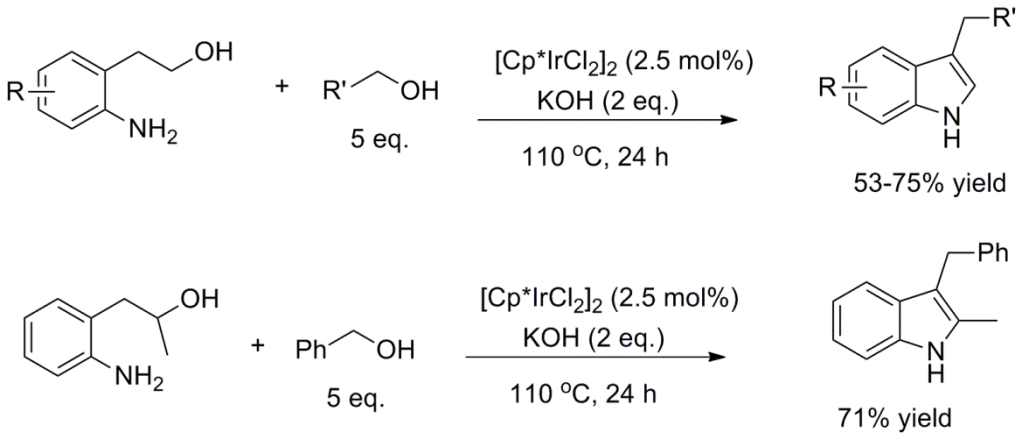
Scheme 89. Ruthenium catalyzed reaction between anilines and 1,2-diols.

The reaction proved to be difficult if ethylene glycol itself was utilized and therefore the authors examined an intramolecular reaction between an alcohol and an amine to provide 2,3-unsubstituted indoles (scheme 90).[67,68] Yamaguchi and co-workers have shown that also an iridium catalyst ($[Cp^*IrCl_2]_2$) is capable of catalyzing the same transformation under very similar reaction conditions (scheme 90).[60]



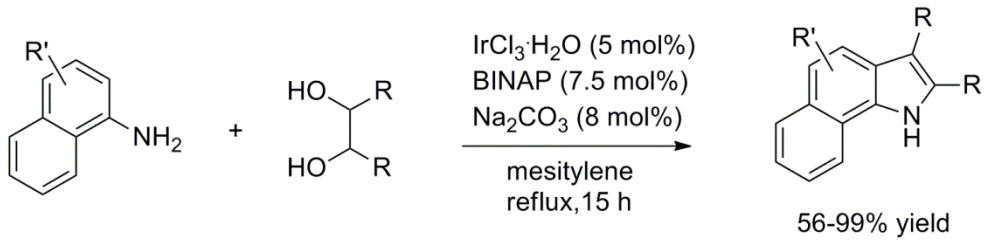
Scheme 90. Metal catalyzed intramolecular reaction between amine and alcohol.

Keep and co-workers have used $[Cp^*IrCl_2]_2$ in the two-component syntheses illustrated in scheme 91 providing either 3-substituted or 2,3-disubstituted indoles.[69] The major drawbacks of this procedure are the great excess of the primary alcohol as well as excess amount of basic additive.



Scheme 91. Two component synthesis catalyzed by $[Cp^*IrCl_2]_2$.

An iridium-BINAP catalytic system can be used for the reaction of naphtylamines with diols to give benzoindoles in good to high yields (scheme 92).[192]



Scheme 92. Use of an iridium-BINAP catalytic system.

4.1.6 Concluding remarks

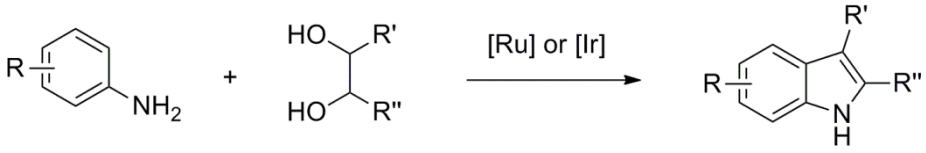
The total number of approaches to indole synthesis is enormous and there is not one specific method that stands out. The classic methods, some dating back more than a century, are still widely used on an industrial scale. However, these methods do not necessarily represent the most environmentally benign procedures. Many of the described methods suffer from disadvantages such as use of toxic starting materials, poor atom economy and poor E-factors. Recently, a growing interest in catalytic procedures has been seen. Environmentally benign syntheses of indoles from readily available starting materials with a broad range of substitution patterns have still not been fully explored.

4.2 Aim of the project

Despite the many known procedures for indole syntheses there is still room for improvements in an environmentally benign fashion. In particular, indoles synthesized from anilines and diols are of great interest.

4.3 Results and discussion

It was speculated that iridium and ruthenium would be able to catalyze both the C-N formation and the C-C formation when anilines and 1,2-substituted vicinal diols are condensated to 2,3-disubstituted indoles (scheme 93).



Scheme 93. Indoles from anilines and vicinal diols.

4.3.1 Previous work within the group

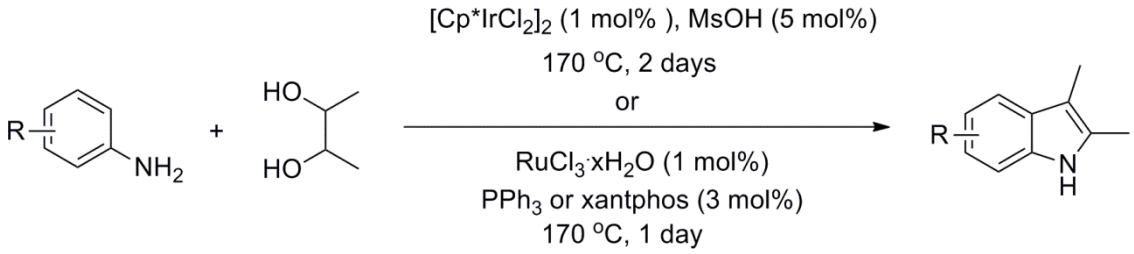
The project was started by former PhD student Matyas Tursky and bachelor student Lasse B. Olsen.

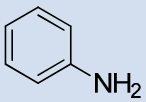
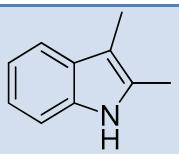
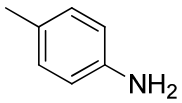
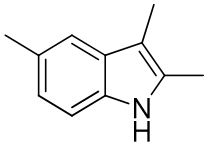
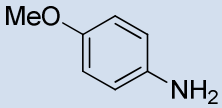
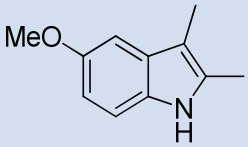
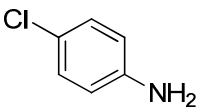
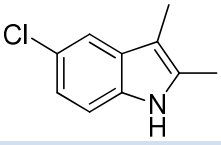
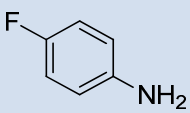
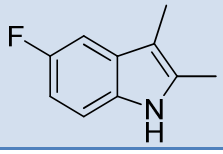
Initially, Matyas Tursky investigated the reaction of aniline and 2,3-butanediol with 1 mol% $[Cp^*IrCl_2]_2$ at 170 ^oC. Screening of additives revealed that a catalytic amount of acid was required and 5 mol% of methanesulfonic acid afforded the highest yield. Neither base nor the absence of an additive was able to co-catalyze the formation of the desired product. The optimal reaction time was established to be 48 hours. As ruthenium catalysts are generally much cheaper than $[Cp^*IrCl_2]_2$ focus was moved to examination of these. As a consequence of previous work within the Madsen group regarding C-C bond formation catalyzed by $RuCl_3$ and PPh_3 (alkylation of the 3-position of oxindoles with alcohols)[193] focus was merely on $RuCl_3$ and different phosphine ligands. Matyas Tursky quickly established that the addition of acid was not necessary as the absence of this provided the indole in 60% yield compared to less than 50% yield in the presence of an acid. The best results were obtained with the monodentate ligand triphenylphosphine and the bidentate ligand xantphos. Interestingly, inconsistent yields of repeated reactions were observed during the screening of ligands. It was speculated that it is due to the

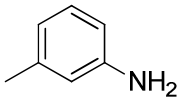
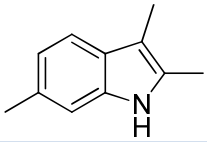
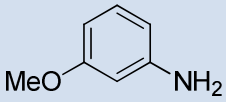
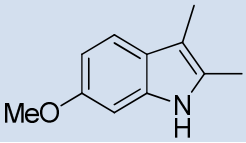
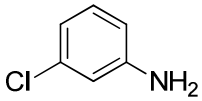
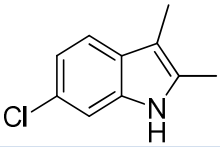
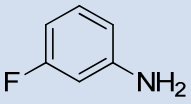
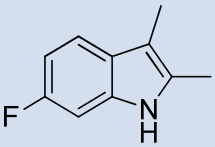
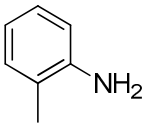
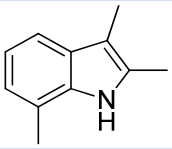
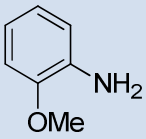
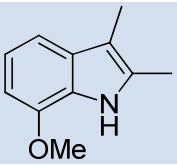
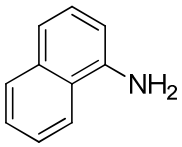
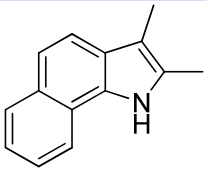
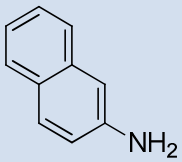
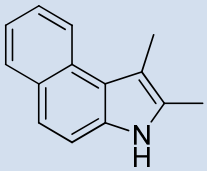
difference in size of the $RuCl_3$ crystals as the preformed catalyst $RuCl_2(PPh)_3$ did not give inconsistent results. A solution to the problem was heating of the reaction mixture to 110 ^oC for one hour and then increasing the temperature to 170 ^oC for the remainder of the reaction time. At 110 ^oC the active catalyst can be generated and the temperature is not high enough to allow for reaction between amine and alcohol. The ruthenium catalyzed reactions required a reaction time of only 24 hours compared to 48 hours for the iridium catalyzed reactions.

A substrate scope was explored for both the iridium and the ruthenium catalyzed condensations and the results are summarized in table 18.

Table 18. Previous results of reaction between anilines and 2,3-butanediol.



Entry	Aniline	Indole	$[Cp^*IrCl_2]_2$ Yield (%)[a,b]	$RuCl_3/PPh_3$ Yield (%)[a,c]	$RuCl_3$/xantphos Yield (%)[a,c]
1			76	60	71
2			61	48	50
3			59	56	53
4			66	57	69
5			46	45	52

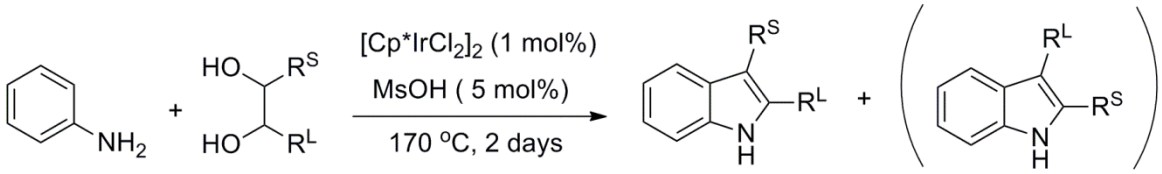
Entry	Aniline	Indole	$[Cp^*IrCl_2]_2$ Yield (%)[a,b]	$RuCl_3/PPh_3$ Yield (%)[a,c]	$RuCl_3$/xantphos Yield (%)[a,c]
6			34	49	51
7			64	48	58
8			47[d]	57[e]	69[e]
9			47	48	52
10			65	72	87
11			41	X	X
12			65	X	X
13			76	X	X

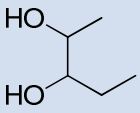
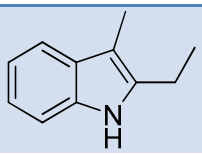
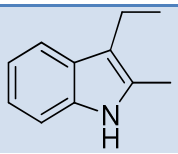
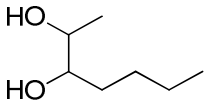
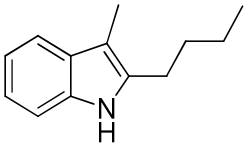
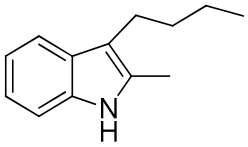
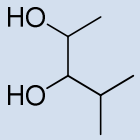
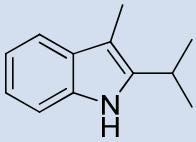
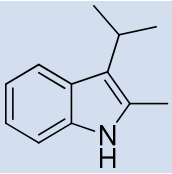
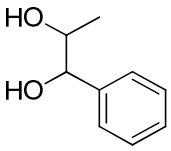
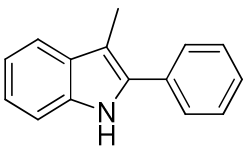
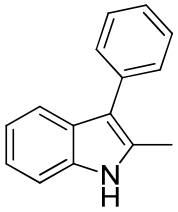
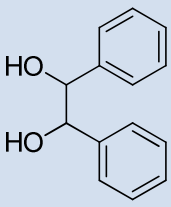
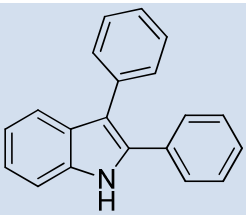
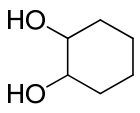
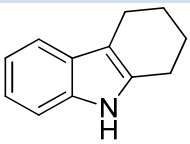
a: after column column chromatography. b: performed by Matyas Tursky; c: performed by bachelor student Lasse B. Olsen. d: isolated as a 4:1 mixture of the 6-chloro and 4-chloro isomer. e: isolated as a 6:1 mixture of the 6-chloro and 4-chloro isomer. ***X****: see section 4.3.2.1.*

The tolerated substituents on anilines were methyl, methoxy, chloro and fluoro (entry 2-11). In general the yields were moderate to good, but problems occurred with chloro and fluoro substituents in the *ortho* position of aniline where less than 25% yield of the corresponding indoles were isolated. In addition also naphtylamines were tolerated (entry 12-13). Unfortunately, bromo, boronic esters, carboxylic acids, methyl esters, cyano, dimethylamino, acetamido, nitro or trifluoromethyl substituents did not afford the desired product as they were either unstable or reacted poorly.

The iridium catalyzed condensation was also examined with unsymmetrical diols in the reaction with aniline by Matyas Tursky and the results are summarized in table 19. The reactions were all highly selective towards the indole with the largest substituent in the 2-position and in general the highest yields were obtained when the substituents on the diol were small.

Table 19. Previous results of reaction between aniline and vicinal diols.



Entry	diol	Indole A	Indole B	Ratio A:B	Yield (%)[a]
1				5:1	80[b]
2				7:1	65[b]
3				1:0	58
4				1:0	31[b]
5			-	-	29
6			-	-	53

a: after column chromatography. b: aniline:diol ratio 2:3.

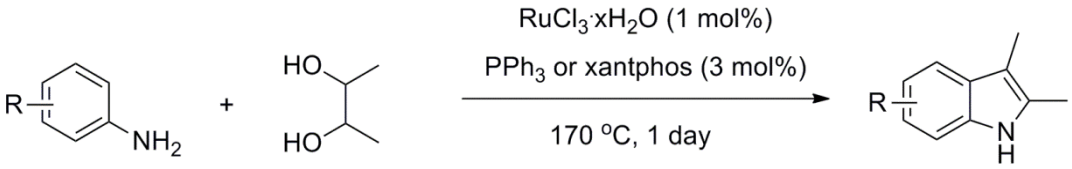
4.3.2 Continuation of the project

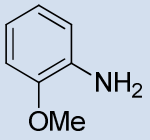
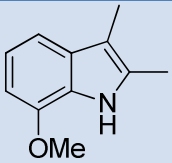
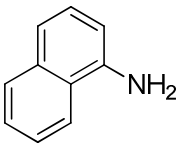
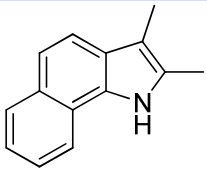
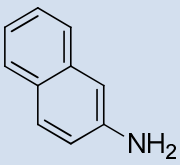
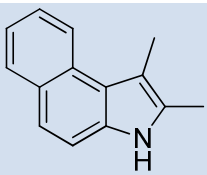
When Matyas Tursky and Lasse B. Olsen left the Madsen group the reaction was not explored in depth in particular in regards to the ruthenium catalyzed condensation of anilines and vicinal diols. Additionally, the mechanism was not fully understood.

4.3.2.1 Reaction of substituted anilines with 2,3-butanediol

The remaining starting materials for the substrate scope studies with 2,3-butanediols and substituted anilines were *ortho*-anisidine as well as 1- and 2-naphtylamine. The results are summarized in table 20.

Table 20. Results of reactions between anilines and 2,3-butanediol.



Entry	Aniline	Indole	$RuCl_3/PPh_3$ Yield (%)[a]	$RuCl_3$/xantphos Yield (%)[a]
1			41	34
2			60	72[b]
3			58[b]	63[a]

a: after column chromatography. a: after 2 days.

Ortho-anisidine was successfully used in the reaction and the corresponding indoles were isolated in 41% and 34% yield with triphenylphosphine and xantphos, respectively (entry 1). These yields are comparable with those of the iridium catalyzed reaction. 1-Naphtylamine afforded the desired indoles in good yields (entry 2). Interestingly, in the reaction employing xantphos as the ligand, full conversion was not obtained after 24 hours, thus the reaction time was increased to 48 hours. 2-naphtylamine afforded the products in good yields and also in these cases an increased reaction time of 48 hours was

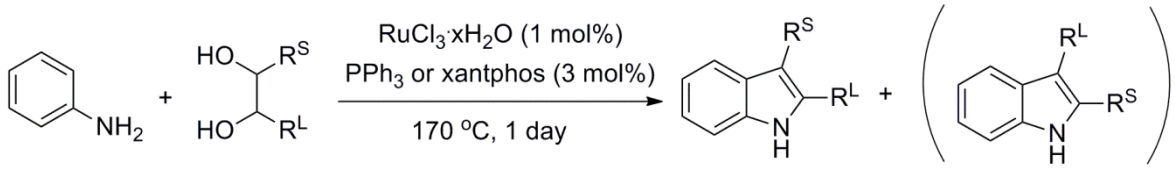
necessary to obtain full conversion of starting materials. The selectivity of the reaction was high as none of the corresponding 2,3-dimethyl-1*H*-benzo[*f*]indole was observed.

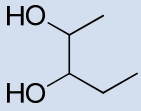
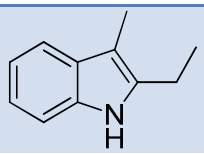
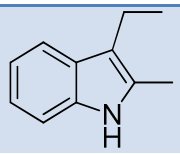
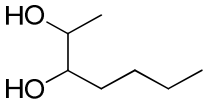
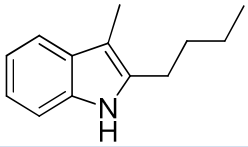
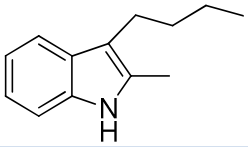
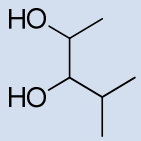
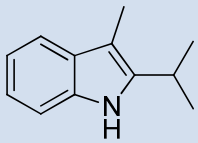
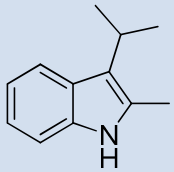
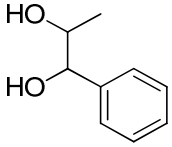
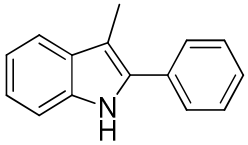
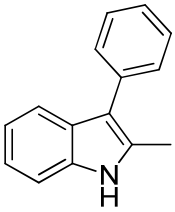
These results combined with those provided by Matyas Tursky and Lasse B. Olsen demonstrate that both $[Cp^*IrCl_2]_2$ and $RuCl_3$ in combination with either PPh_3 or xantphos provides indoles from anilines and diols in comparable yields. The smallest difference in yield for a given substrate is 5 percentage points and the highest is 22 percentage points. There is no clear trend in which of the catalytic systems that are superior.

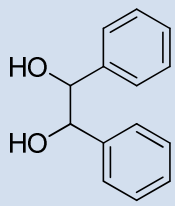
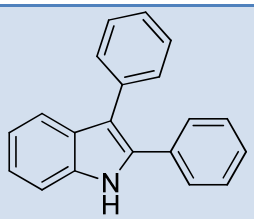
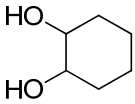
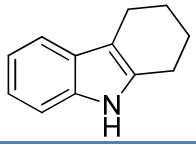
4.3.2.2 Reaction of aniline with unsymmetrical vicinal diols

The unsymmetrical vicinal diols were also tested in the ruthenium catalyzed condensations and the results are summarized in table 21.

Table 21. Results of reactions between aniline and vicinal diols.



Entry	diol	Indole A	Indole B	PPh_3 Yield (%)[a]	Xantphos Yield (%)[a]
1				49 (1:0)[b]	57 (1:0)[b]
2				54 (7:1)[b]	61 (1:0)[b]
3				32 (1:0)[c]	-
4				27 (1:0)[d]	28 (1:0)[d]

5			-	-	-
6			-	50[e]	52[e]

Numbers in parenthesis indicate A:B ratio a: after column chromatography. b: aniline:diol ratio is 2:3. c: after 3 days. d: performed by Lasse B. Olsen. e: with 5% MsOH.

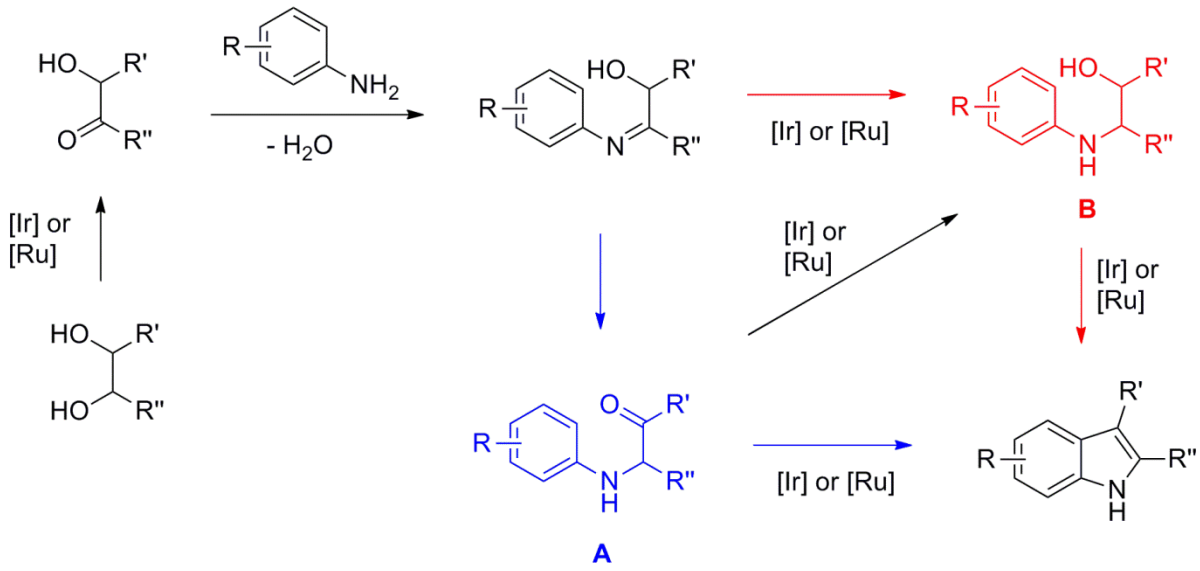
For 2,3-pentanediol the reactions afforded almost solely the indoles with the ethyl substituent in the 2-position in 49% and 57% yield with triphenylphosphine and xantphos, respectively (entry 1). When a longer alkyne chain on the diol was employed (2,3-heptanediol, entry 2) the reaction also provided a small amount of the 3-substituted products. Triphenylphosphine resulted in a total yield of 54% in a 7:1 ratio of 2- and 3-substituted products, respectively. Interestingly, when xantphos was used only the 2-substituted product was observed and this was isolated in 61% yield. Both 2,3-pentane- and 2,3-heptanediol in a slight excess was required in order for the reactions to go to completion. When 4-methyl-2,3-pentanediol was utilized a reaction time of 3 days was necessary to obtain full conversion. Interestingly, only with triphenylphosphine was the reaction successful and the indole with the isopropyl group in the 2-position was isolated in 32% yield (entry 3). Lasse B. Olsen synthesized 2-phenyl-3-methylindole with both triphenylphosphine and xantphos in 27% and 28% yield, respectively (entry 4). Employment of two phenyl substituents was unsuccessful for both triphenylphosphine and xantphos (entry 5). GC-MS analysis revealed the presence of many by-products in large quantities and isolation was therefore not attempted. Similar results were seen for 1,2-cyclohexanediol (entry 6). GC-MS analysis indicated that not only was by-product formation a concern, but also intermediates were observed. However, addition of 5% methanesulfonic acid afforded a clean reaction without the presence of intermediates or by-products and the product was isolated in 50% and 52% yield with triphenylphosphine and xantphos, respectively.

With a few exceptions the yields obtained from ruthenium catalyzed condensation of anilines with unsymmetrical vicinal diols are comparable with those obtained by iridium catalysis. Use of 2,3-pentanediol afforded significantly lower yields, though the selectivity was better and only one product was obtained. Iridium catalysis was to a great extend

superior when an isopropyl substituent as well as the diphenyl substituents were utilized. An explanation for this difference has not been established.

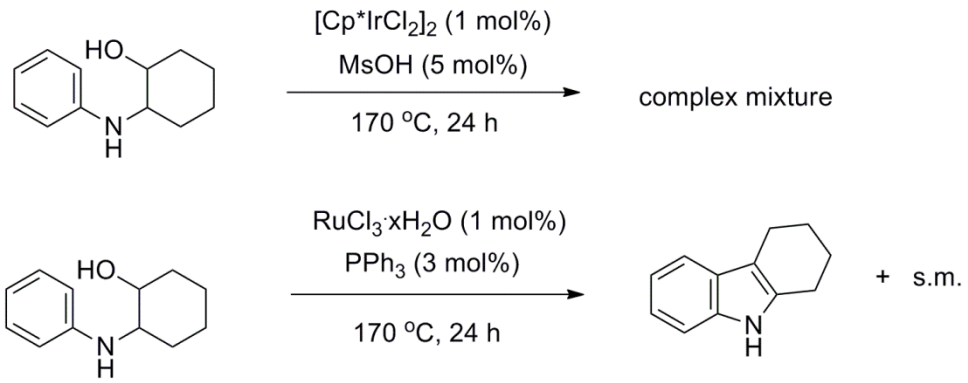
4.3.3 Mechanism

The proposed mechanism is illustrated in scheme 94. The first step is oxidation of the alcohol to the ketone. This is followed by reaction with the amine that upon removal of water affords an α-hydroxyimine. This imine can then undergo either a Voigt reaction to give α-amino ketone (**A**) or catalytic reduction to give the α-amino alcohol (**B**). Either **A** or **B** could possibly furnish the indole directly, or **A** could be reduced to **B** by the catalyst.



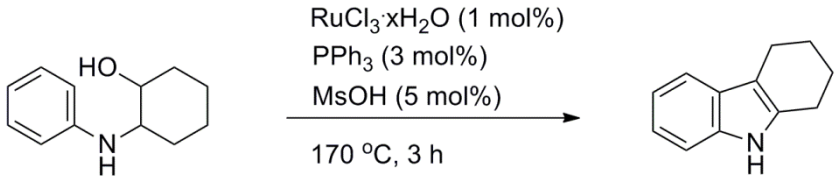
Scheme 94. Possible pathways for indole formation from anilines and vicinal diols.

Mechanistic experiments for both iridium and ruthenium catalyzed reactions were conducted to provide more information about the reaction. The reactions performed with the iridium catalyst were conducted by Matyas Tursky who also synthesized α-amino alcohol **B**. This was reacted under the iridium or ruthenium reaction conditions (scheme 95). With iridium catalysis the result was a complex reaction mixture with only little formation of the indole. The ruthenium-triphenylphosphine catalytic system allowed the formation of the product but still after 24 hours a substantial amount of starting material remained. This concludes that α-amino alcohol **B** is not a likely intermediate in the indole formation from anilines and diols.



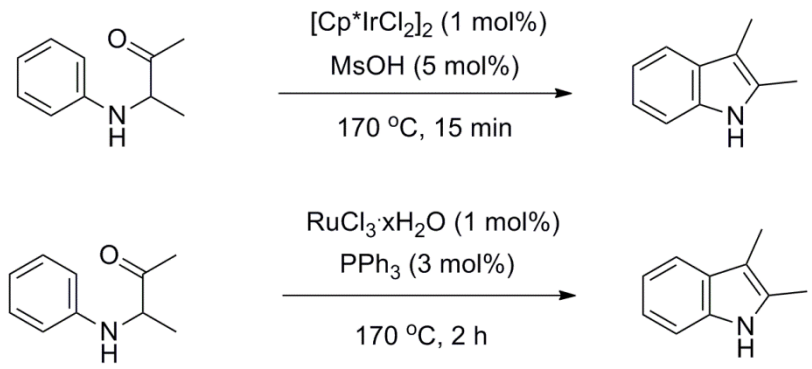
Scheme 95. Mechanistic experiments for route B.

As it was found that methanesulfonic acid improved the reaction outcome in one case for a ruthenium catalyzed reaction, an experiment of α-amino alcohol **B** with ruthenium and triphenylphosphine and 5% of the acid was performed (scheme 96). After only 3 hours the product was the major peak in the GC-MS chromatogram. This indicates that the acidid additive has an influence on the reaction (at least with the more difficult substrates).



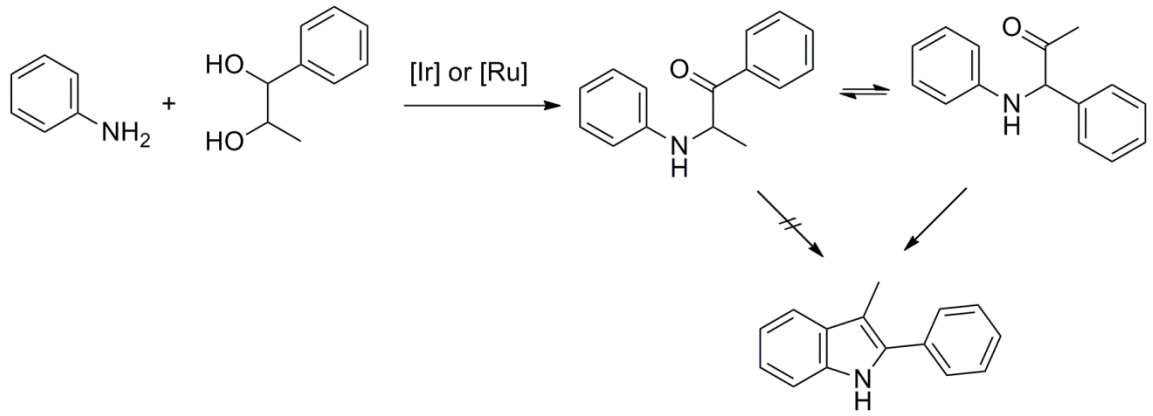
Scheme 96. Influence of MsOH in the ruthenium catalyzed reaction.

Also α-amino ketone **A** was prepared and reacted under both iridium and ruthenium reaction conditions (scheme 97). This resulted in full conversion to the indole in 15 min and 2 hours with iridium and ruthenium, respectively. This clearly supports the postulate that α-amino ketone **A** is an intermediate in the reaction; hence the Voigt reaction plays an important role in the formation of the indoles from anilines and vicinal diols.

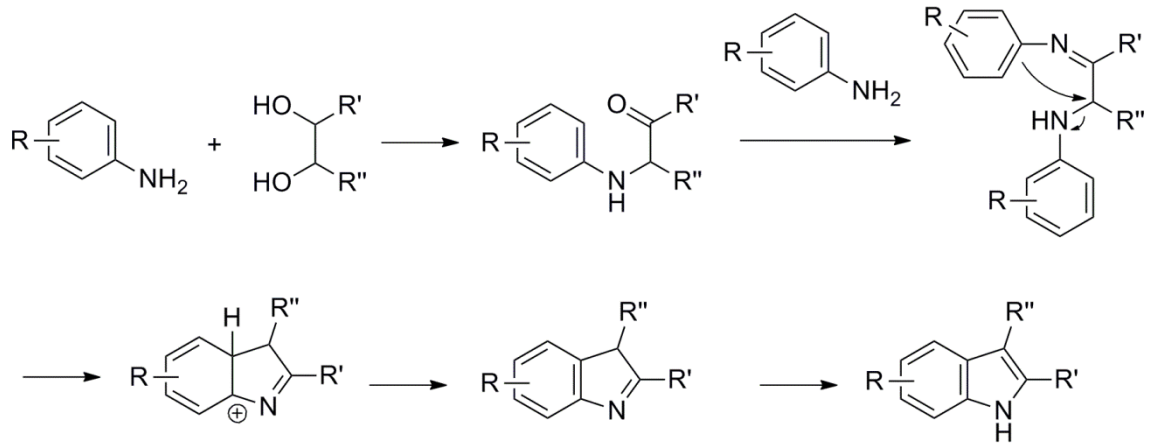


Scheme 97. Mechanistic experiments for route A.

In this work there has not been conducted any mechanistic experiments to establish the explanation for the high selectivity towards the indole with the largest substituent in the 2-position (scheme 98). It is merely speculated that an isomerization similar to that seen in the Bischler indole synthesis[168,194,195] is the underlying cause (scheme 99).



Scheme 98. Selectivity of the reaction with unsymmetrical diols.



Scheme 99. Bischler mechanism of indole formation.

It should be noted that with the mechanistic results reported herein it is not clear if the iridium and the ruthenium catalyzed reactions proceed via the exact same pathway. The mechanistic evidence for the iridium catalyzed reactions is to some extend stronger towards the pathway via α-amino ketone **A**, whereas the results for the ruthenium catalyzed reactions could indicate an alternative pathway.

4.4 Conclusion

Indoles have been synthesized from anilines and vicinal diols by $RuCl_3$ and triphenylphosphine or xantphos catalysis in good yields. The procedure is highly atom economical as neither solvent nor additives are required and water and dihydrogen are the only by-products. When unsymmetrical diols are employed the corresponding indole is favoured with the largest substituent in the 2-position and it is believed to follow the Bischler mechanism. The reaction is best suited for diols with small substituents. When larger substituents such as cyclohexyl, isopropyl and phenyl are employed yields drop radically and longer reaction times are in some cases required. However, it was briefly explored that methanesulfonic acid increases reaction rate and hampers by-product formation. Unfortunately, due to time restraints it was not possible to test that for all the different substrates. Mechanistic experiments strongly supports that for the ruthenium catalyzed reactions the Voigt reaction plays an important role.

5 Protein folding

This part of the thesis describes research conducted at The Scripps Research Institute, La Jolla, California, USA as a part of the PhD programme. The project was supervised by Professor Philip E. Dawson and concerns a better understanding of protein folding.

5.1 Background

Protein folding is a very complex procedure, and it is one of the most perplexing problems in molecular biology. It can be divided into two parts;[196] 1) how is it possible to predict the three-dimensional structure of the biologically active compound from the amino acid sequence? and 2) how does the protein reach this three-dimensional structure? In 1969 Levinthal pointed out, that even if a small protein would have to go through all possible conformations to reach the active structure it would take longer than the age of the universe.[197] Consequently, proteins *must* be programmed for efficient folding.

5.1.1 Folding mechanisms

Protein folding can be compared to a chemical reaction; a starting material (the unfolded, denatured protein), transition states (maybe also intermediates) and then the product (the folded, native protein). The reaction of protein folding does not involve breaking and forming new chemical bonds. Instead, it involves breaking and formation of non-covalent interactions, most often hydrogen bonding. Each individual interaction is very small, but due to the very large number of interactions the total energy is large. Interestingly, the folded protein is only slightly more stable compared to the denatured state (generally by 5-15 kcal/mol).[198]

The fundamental description of protein stability is the difference in free energy, ΔG, between the unfolded and folded states, which is defined as:[199]

$$\Delta G = \Delta H - T \cdot \Delta S \quad \text{(eq. 1)}$$

where ΔH is the enthalpy difference, T the temperature and ΔS the entropy difference. A positive value of ΔG implies that the folded state is favoured.

In the denatured state the protein has some configurational preferences and is therefore never fully unfolded. As a consequence of this, it is rather termed the denatured state

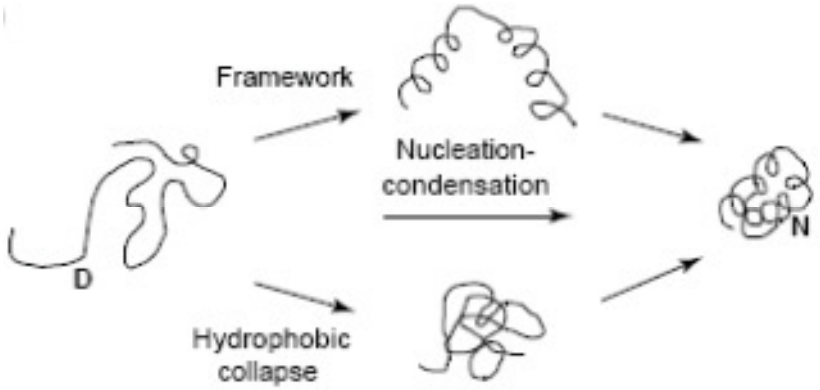
instead of unfolded state in order to minimize confusion. In the denatured state many of the side groups can rotate and therefore the configurational entropy of this state is high. However, in the folded stated the structure is more locked and the side groups have limited ability to rotate resulting in low configurational entropy. In order for the folding reaction to favour the folded state the decrease in entropy has to be balanced by a gain in enthalpy.[198]

Recently, protein folding has gained an increased attention as a result of the growing interest in diseases resulting from protein misfolding and aggregation.[196] The mechanism of protein folding is not fully understood and in particular the role of local interactions during the process is in focus. Initially, it was believed that backbone hydrogen bonding was the driving force of folding.[200] This was replaced by the theory that the hydrophobic effects initiate folding[201], which resulted in numerous studies concerning side-chain interactions.[202-204] However, there is currently an ongoing debate concerning which factors contribute the most to protein folding. This has lead to many different theories, e.g.:

- Jigsaw model – where a large number of parallel folding pathways are present.[205]
- Framework model – where the secondary structures are initially formed followed by docking of these to furnish the folded structure.[206-209]
- Hydrophobic collapse model – where hydrophobic effects allow the protein to collapse followed by formation of the secondary structures.[201,210-212]
- Diffusion-collision model – where formation of subdomains that adopts native conformations initiates the process followed by a collision to afford the folded structure.[213-215]

Examinations of several well-known proteins have ruled out these models one by one.[216,217] As described above a protein is never fully unfolded and there is to some extent a residual structure, which might not be native-like, but something that guides folding. As a consequence of this, it is now believed that the mechanism of folding lies somewhere between the framework and hydrophobic collapse models. This is called the nucleation-condensation (or nucleation-collapse) model, where secondary and tertiary structures are formed more or less simultaneously (figure 9).[217]

Figure 9. Nucleation-condensation model.[217]



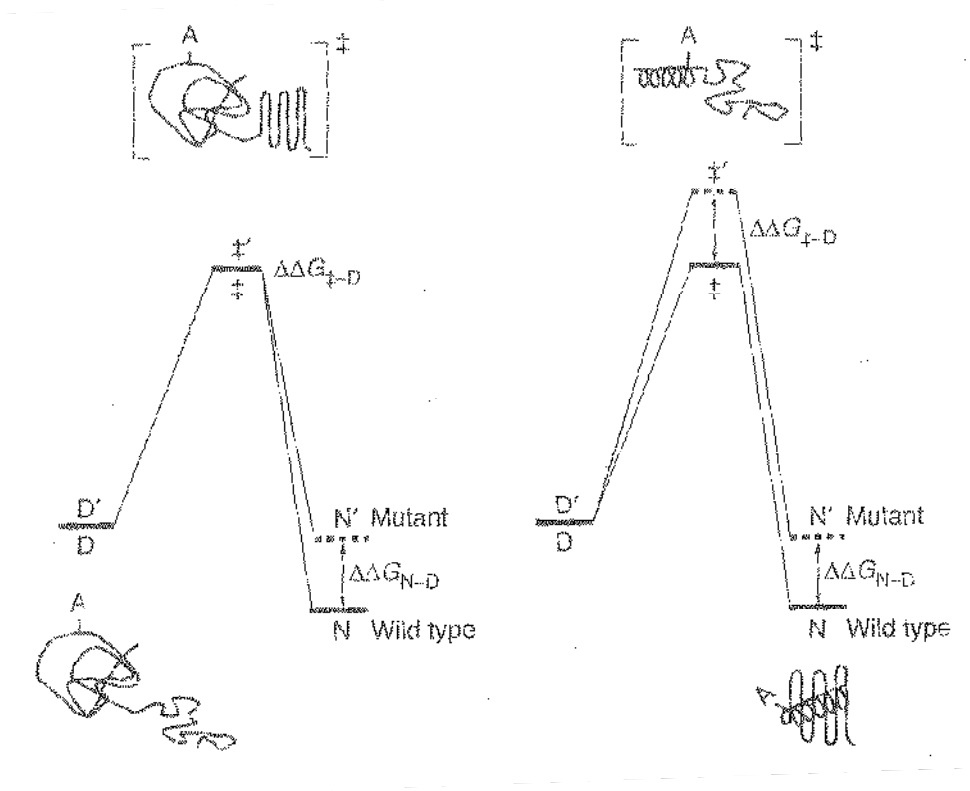
It is possible to view the framework and hydrophobic collapse models as extremes of the nucleation-condensation model.[217]

5.1.2 The protein engineering method for transition state analysis

As a result of the complexity of protein folding it is essential that theoretical and experimental studies are combined to obtain a better understanding of the procedure. One approach is to combine theoretical molecular dynamics, NMR and experimental transition state analysis.[196] Herein, the latter will be described.

The protein engineering method developed by Fersht and co-workers is used to study the transition states of protein folding.[218] It involves thermodynamic and kinetic measurements of folding and unfolding of mutants in comparison with the wild type protein. The method is also called Φ-value analysis or transition state analysis, and it is the only method for transition state analysis at an almost atomic level.[217] Figure 10 illustrates two different pathways of folding of the denatured protein (D) to the native structure (N) and the associated free energie diagrams. Residue A represent the site of mutation (mutant is denoted by ‘) and if this is a part of the α-helix in the folded structure transition state analysis will reveal if the helix is or is not formed (or partially formed) in the tranisition state.

Figure 10. Free energy diagrams for protein folding.[198]



The thermodynamic measurements provide values for the change in stability of the protein upon mutation, $\Delta\Delta G_{N\text{-}D}$, and the kinetic measurements provide values for the change in stability of the transition state upon mutation, $\Delta\Delta G_{‡\text{-}D}$. Identical values represent that the region where the mutation site is located is equally folded in the transition state and in the folded structure. If $\Delta\Delta G_{‡\text{-}D}$ is zero it means that the region is equally unfolded in the transition state and in the denatured state. The relationship between $\Delta\Delta G_{N\text{-}D}$ and $\Delta\Delta G_{‡\text{-}D}$ is described as the Φ-value:[219]

$$\Phi = \frac{\Delta G_{‡\text{-}D} - \Delta G'_{‡\text{-}D}}{\Delta G_{N\text{-}D} - \Delta G'_{N\text{-}D}} \frac{\Delta\Delta G_{‡\text{-}D}}{\Delta\Delta G_{N\text{-}D}} \quad \text{(eq. 2)}$$

In other words, if Φ = 0 it implies that the site of mutation is completely denatured in the transition state and therefore the mutation does not affect the folding rate. In terms of energy it means that the interaction the residue makes in the native state is lost in the transition state. If Φ = 1 the site of mutation is completely folded in the transition state and the transition state loses as much energy as the folded state. It also indicates that the interaction, which the residue makes, is retained in the transition state. Fractional or intermediate values of Φ are more difficult to interpret as there are no linear relationship

between the value and formation of structure. There are several different reasons for fractional values of Φ depending on the exact nature of the protein, e.g. parallel folding pathways, access of water to the mutation site.[218] Fortunately, for the protein selected for this project fractional values arrise from weakened interactions.[198] For example a Φ-value of 0.6 indicates that 60% of the interactions that are present in the folded state are formed in the transition state, e.g. if it concerns is an α-helix, this is partially formed in the transition state.[198] If $\Phi > 1$ it could imply that the side chain makes more interaction in the transition state compared to the native state.[216]

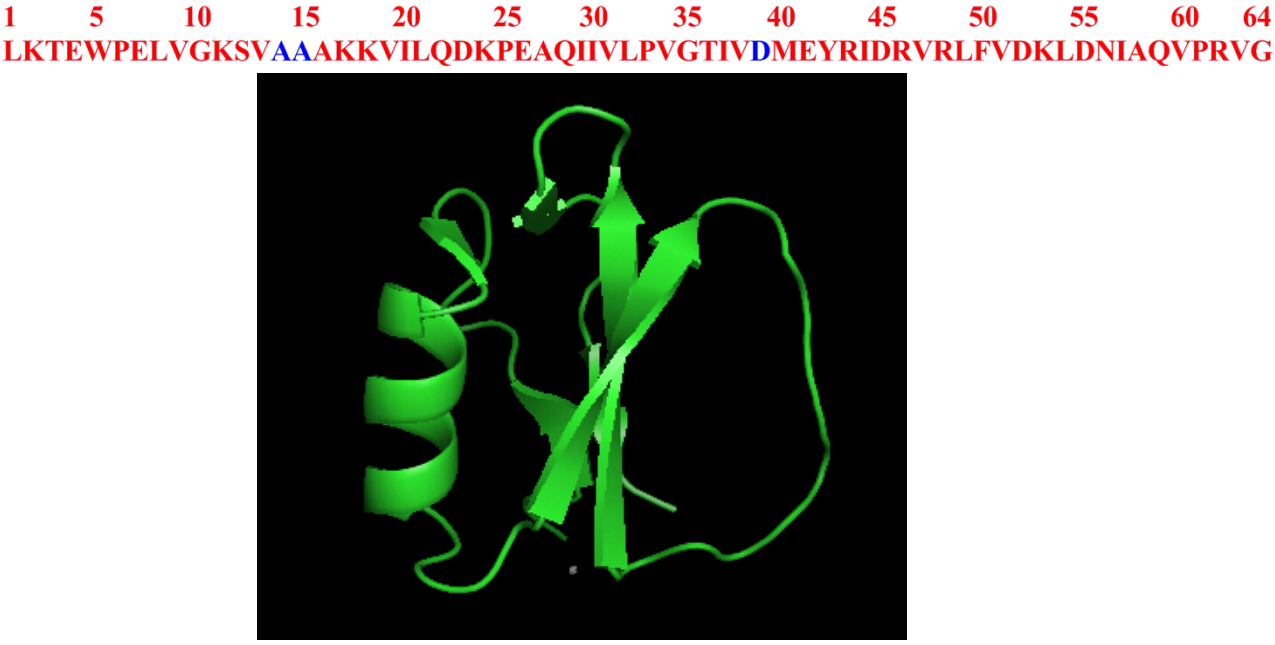
It should be noted that figure 10 could give the impression that there is no energy difference between the denatured states of wild type protein and the mutant. This is of course not always the case. However, the difference in energy for the denatured states cancels out in the equations since all energy differences are relative to the denatured state.[198]

More detailed description on how $\Delta\Delta G_{N\text{-}D}$ and $\Delta\Delta G_{‡\text{-}D}$ are calculated from the results of the thermodynamic and kinetic experiments are provided in section 5.3.

5.2 Chymotrypsin inhibitor (CI2)

One of the most well known proteins in terms of transition state analysis is chymotrypsin inhibitor (CI2). It is a very unique protein because it folds via very simple two-state kinetics without accumulation of stable intermediates.[196] It is found in the albumin fraction of seeds from the *Hiproly* strain of barley and is a member of the potato inhibitor I family of serine protease inhibitors.[220] The sequence and 3D structure are show in figure 11.

Figure 11. Amino acid sequence and 3D structure of CI2.

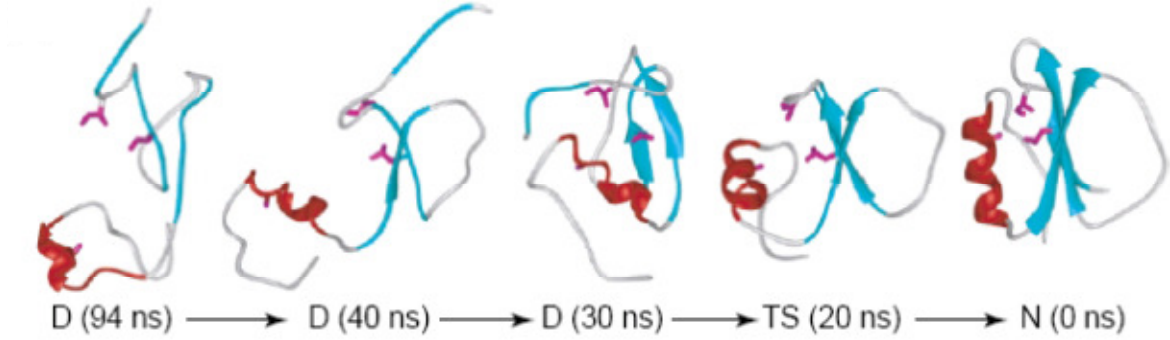


The complete amino acid sequence of CI2 amounts to 83 amino acids. However, the structure in figure 11 contains only 64 amino acids, since most studies of CI2 are lacking the first 19 amino acids. When the structure of CI2 was determined in 1987 only residue 20 to 83 could be determined by NMR and X-ray crystallography.[221] Later, it was found that the first 19 residues are highly disordered and do not contribute to either the stability or the activity of the protein.[216] The protein is a mix of β-strands and one α-helix. The α-helical region, which is the focus of this work, involves residues 12 to 24. It should be noted that the amino acid sequence has been slightly modified. It concerns the residues marked in blue in the sequence (figure 11). Residues 14 and 15 are altered from glutamic acids to alanines in order for crystals to grow easier, which are necessary for X-ray crystallography.[222] Residue 39 is changed from threonine to aspartic acid for easier synthesis of the protein. None of these changes affect the folding mechanism[218] and herein this structure is denoted wild type CI2.

5.2.1 Folding of CI2

A combination of molecular dynamic simulations, NMR and transition state analysis of several CI2 mutants has led to the suggested folding pathway provided in figure 12.[196] It implies that there are many states of the denatured protein and it is more like a random coil with some native, residual helical structure. The residues marked in pink are those important to folding of the nucleus.[217]

Figure 12. Simulations of unfolding of CI2 in reverse time.[217]



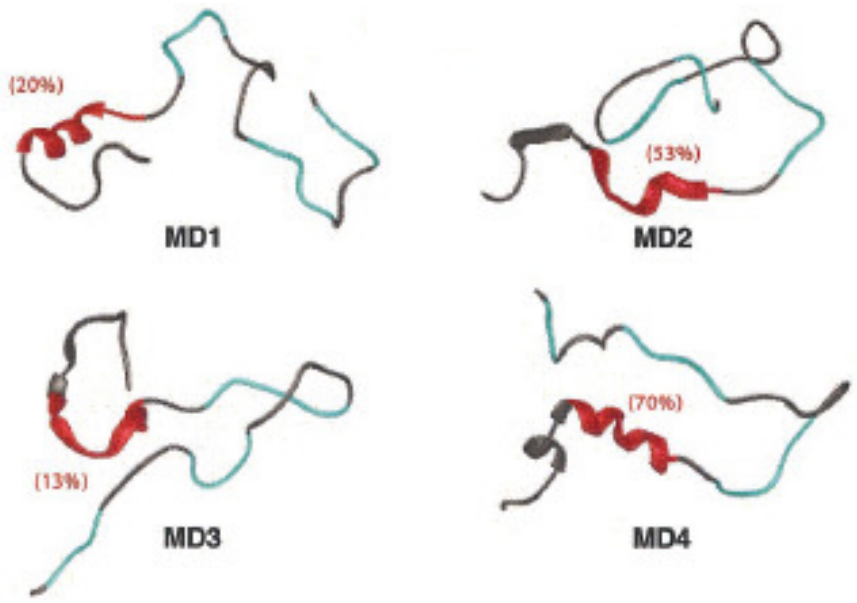
As previously mentioned CI2 folds via a two-state model, where only the denatured structure, transition state and native structure occupy free energy minima. It should be noted that it does not mean that intermediates are not present only that they are not significantly present.[218] Molecular dynamic simulations have identified one intermediate, which facilitates folding, but unfortunately it is has not been supported by any experiments.[196] Consequently, this proposed intermediate is ignored in the studies of CI2 folding.

The first step of the nucleation-condensation mechanism for CI2 reported by Fersht and co-workers[218] is the formation of the nucleus (the α-helix), and subsequently the overall structures condense around the nucleus. The protein must be "programmed" via a rapid random search of conformations under conditions that favour folding to first shape the α-helix. However, it is not fully formed and the interactions are only weak. This structure is the transition state and can be termed as a "native-like" structure. The rest of the protein condenses around the helix via secondary and tertiary interactions. A well established characteristic of this model is that the nucleation site does not necessarily have to be well established in the denatured state as it might be in the process of being formed in the transition state.[218,223] Additionally, long range interactions are more important than the more local interactions. In other words the α-helix and β-sheets cannot be formed without long range interactions.[218]

Another factor to take into consideration is that despite the low levels of residual structure in the denatured state, there are still some conformational preferences. Consequently, not all conformations exist in the denatured state. As previously described the protein does not go through all possible conformations to reach the active structure. There is a loose retention of native topology that together with a great deal of variation of secondary structure and side-chain interactions makes the protein obtaining the

confirmation of the transition state.[196] The denatured state of CI2 is no exception. It is highly expanded and as previously described almost a random coil with some residual structure. Simulations of the denatured states are illustrated in figure 13 and it is clear that the α-helix is partially formed together with a small amount of hydrophobic clustering.[224]

Figure 13. Simulations of the denatured states of CI2.[224]

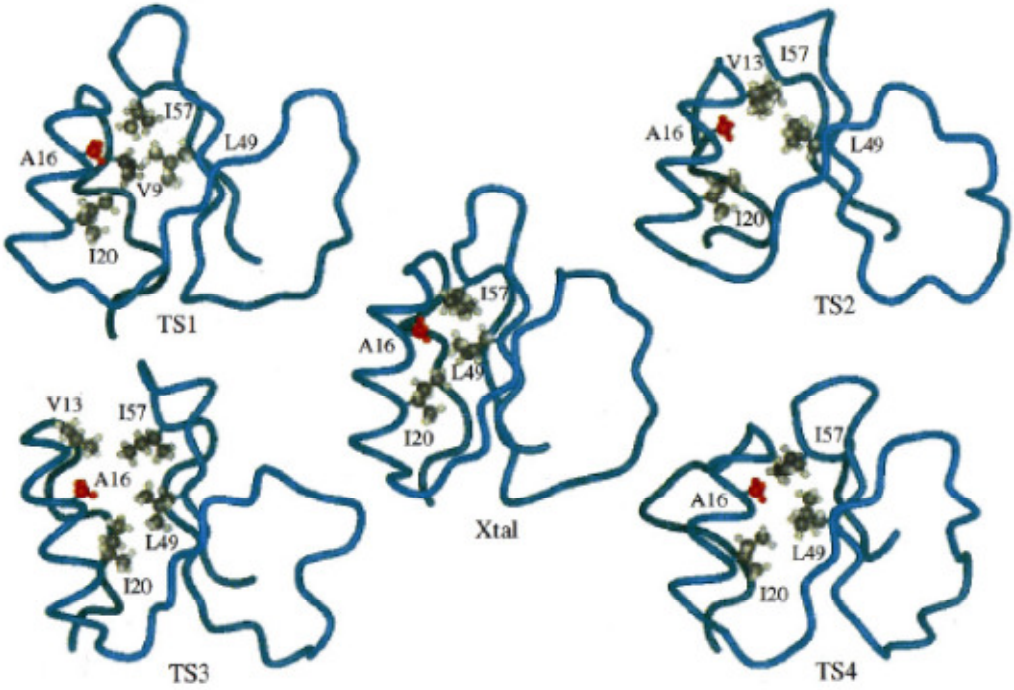


The advantage of studying a protein with a simple folding mechanism is that the single rate-determining transition state can be studied in either of the directions of folding and unfolding.[218] The nucleation-condensation model has since also been used to study other systems as it has proved to be a quite common mechanism.[196]

5.2.2 Transition state analysis of CI2

It has been demonstrated that the nucleus consists of those residues that have the highest Φ-values.[198] The most extensive study on CI2 mutants has been conducted by Fersht and co-workers with a total of 99 mutants.[218] The results supports that the α-helix is to some extent formed in the transition state. The study revealed that only two residues in the core of the protein resulted in Φ-values above 0.5; residue 16 (alanine) and 49 (leucine). These two residues together with the surrounding residues represent the nucleus and when the interactions are made the rest of the protein condenses around it. The crystal structure of native CI2 and the simulations of the transition state are illustrated in figure 14.[219]

Figure 14. Crystal structure of CI2 (Xtal) and simultations of transition states of folding (TS1-4).[219]



Φ-values of the remainder of the residues in the α-helical region were 0.5 for residues 12-18 and for residues 19-24 the values ranged from -0.4 to 0.2. Negative Φ-values were predominantly obtained when residues were mutated to alanine. Alanine increases the preference to form the α-helix as it has the highest α-helical propensity out of the 20 naturally occurring amino acids. It results in stabilization of the transition state and destabilization the less helical denatured state and overall this affords negative Φ-values.

The authors concluded that only the N-terminal part of the helix is formed in the transition state. Interestingly, the data is in disagreement with NMR studies and molecular dynamics that implies that it is rather the C-terminus that initiates folding of the α-helix.[224] It could be reasoned that the side-chain mutations applied by Fersht and co-workers are not suitable due to the fact that the C-terminus residues do not make contacts with the core of the protein. It is also possible that the α-helix in the transition state is more folded than it is in the native state. This is supported by the crystal structure and NMR studies of CI2 that revealed a bending of the helix and long hydrogen bonds on the solvent exposed phase.[224]

Previous work performed in the Dawson group has demonstrated that backbone hydrogen bonding is not a requirement for protein folding.[225] CI2 mutants with some of the amide bonds replaced with ester bonds were prepared. This produced protein was

still able to fold, however the protein was not as stable as the all amide wild type CI2. In more details the mutations led to Φ-values of 0.6 and 0.7 for residue 19 and 22, respectively, suggesting that backbone hydrogen bonds at the C-terminus are formed to a significant degree in the transition state (unpublished results in the Dawson group). This is in agreement with the NMR and molecular dynamic studies[224] mentioned above and do not agree with the results obtained from Φ-value analysis by Fersht and co-workers.[218]

5.3 Calculation of Φ-values

In this section it is described how to calculate the Φ-values. As previously mentioned Φ is defined as:

$$\Phi = \frac{\Delta\Delta G_{\ddagger\text{-D}}}{\Delta\Delta G_{\text{N-D}}} \qquad \text{(eq. 2)}$$

The Gibbs free energies are calculated from thermodynamic and kinetic measurements of folding and unfolding.

5.3.1 Guanidine hydrochloride denaturation monitored by fluorescence

Guanidine hydrochloride denaturation experiments[226] can be used to calculate the difference in stability of a protein upon mutation, $\Delta\Delta G_{\text{N-D}}$, as well as the difference in stability of the transition state upon mutation, $\Delta\Delta G_{\ddagger\text{-D}}$. The chosen technique to analyse guanidine denaturation is measurement of the fluorescence emission. Other techniques include UV difference spectroscopy, circular dichroism, optical rotation and NMR.[226] CI2 is very simple to analyse by guanidine hydrochloride denaturation due to a great change in the physical properties of the protein as it unfolds. Additionally, this procedure requires less amount of protein compared to the other techniques.[226] In a fluorescence experiment the molecule is excited at a given wavelength and after relaxation it emits light at a different wavelength. The preferred residue for excitation is tryptophan, which fortunately there is one of in CI2. The emission by tryptophan is in some proteins highly quenched in the native state and as the protein unfolds a large increase in the fluorescence intensity is detected.[227] For other proteins like CI2 it is the opposite. The nearby lysine residue of tryptophan in CI2 quenches the emission in the folded state. Other requirements for Guanidine denaturation experiments include:[226]

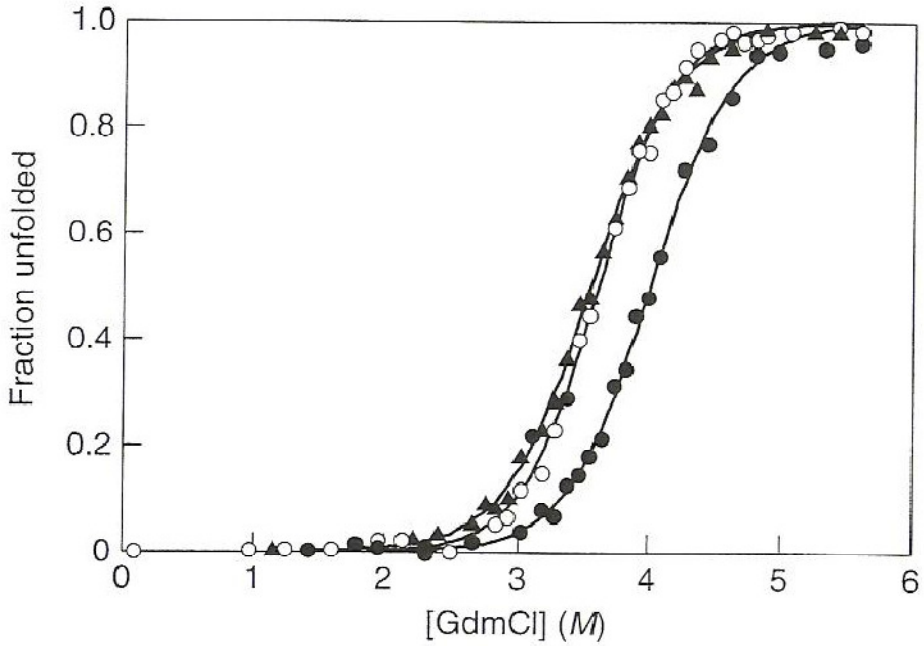
- A minimum of 10-15 data points are required to be able to get reliable data from the measurements.
- It is crucial that equilibrium have been reached before the measurement is made. For some proteins it can take days to reach equilibrium.
- It is important to know if the unfolding process is reversible. Irreversible folding is most common for proteins containing free sulfhydryl groups because these form stable disulfide bonds.
- Aggregation must be avoided.

It has been shown by Fersht that the guanidine denaturation of CI2 is completely reversible and equilibrium is reached within one hour.[220]

5.3.2 Thermodynamic experiments

The data obtained from thermodynamic measurements of guanidine hydrochloride denaturation are fluorescence versus the guanidine hydrochloride concentration. A typical denaturation curve is shown in figure 15.

Figure 15. Guanidine hydrochloride denaturation curves for CI2.[198]



These curves are the basis for calculations of the free energies of the unfolding reactions.[216,218,220] The free energy at a particular denaturant, guanidine hydrochloride, concentration [GdnHCl] is defined as:

$$\Delta G_{\mathrm{D-N}}^{[GdnHCl]} = -RT \ln K_{\mathrm{D\text{-}N}} \quad \text{(eq. 3)}$$

where R is the gas constant, T is the temperature and $K_{\mathrm{D\text{-}N}}$ is the equilibrium constant for the unfolding reaction. In a two-state model only the denatured and native structures are populated:

$$frac_{\mathrm{N}} + frac_{\mathrm{D}} = 1 \quad \text{(eq. 4)}$$

where $frac_{\mathrm{N}}$ and $frac_{\mathrm{D}}$ is the fraction of protein folded and unfolded, respectively. The observed fluorescence, F, is given by:

$$F = F_{\mathrm{f}} \cdot frac_{\mathrm{N}} + F_{\mathrm{D}} frac_{\mathrm{u}} \quad \text{(eq. 5)}$$

where F_{N} and F_{D} are the fluorescence of the folded and unfolded protein, respectively. Combining equation 4 and 5 gives:

$$frac_{\mathrm{D}} = \frac{F_{\mathrm{N}} - F}{F_{\mathrm{N}} - F_{\mathrm{D}}} \quad \text{(eq. 6)}$$

The equilibrium constant for folding is defined as:

$$K_{\mathrm{D-N}} = \frac{frac_{\mathrm{D}}}{1 - frac_{\mathrm{D}}} = \frac{frac_{\mathrm{D}}}{frac_{\mathrm{N}}} = \frac{F_{\mathrm{N}} - F}{F - F_{\mathrm{D}}} \quad \text{(eq. 7)}$$

It is assumed that F_{N} and F_{D} are linearly dependent on guanidine hydrochloride concentration:

$$F_{\mathrm{N}} = \alpha_{\mathrm{N}} + \beta_{\mathrm{N}} \cdot [\mathrm{GdnHCl}] \quad \text{(eq. 8)}$$

$$F_{\mathrm{D}} = \alpha_{\mathrm{D}} + \beta_{\mathrm{D}} \cdot [\mathrm{GdnHCl}] \quad \text{(eq. 9)}$$

Consequently, the observed fluorescence is derived from a combination of equation 7, 8 and 9:

$$F = \frac{F_N + F_D \cdot K_{D-N}}{1 + K_{D-N}} = \frac{(\alpha_N + \beta_N \cdot [\text{GdnHCl}]) + (\alpha_D + \beta_D \cdot [\text{GdnHCl}]) \cdot \exp\left(\frac{\Delta G_{D-N}^{[GdnHCl]}}{-R \cdot T}\right)}{1 + \exp\left(\frac{\Delta G_{D-N}^{[GdnHCl]}}{-R \cdot T}\right)}$$

(eq. 10)

where $\Delta G_{D-N}^{[\text{GdnHCl}]}$ is the free energy of unfolding at a particular denaturant concentration. Additionally, it is assumed that there is a linear relationship between the free energy of unfolding in the presence of guanidine hydrochloride and the concentration of denaturant. The free energy of unfolding is therefore:

$$\Delta G_{D-N}^{\text{GdnHCl}} = \Delta G_{D-N}^{H_2O} - m_{D-N}[\text{GdnHCl}] \quad \text{(eq. 11)}$$

where $\Delta G_{D-N}^{H_2O}$ is the free energy of unfolding in water (zero molar guanidine hydrochloride) and m_{D-N} is a constant that is proportional to the increase in the degree of exposure of the protein on denaturation. The observed fluorescence is derived by combining equation 10 and 11:

$$F = \frac{F_N + F_D \cdot K_{D-N}}{1 + K_{D-N}} =$$

$$\frac{(\alpha_N + \beta_N \cdot [\text{GdnHCl}]) + (\alpha_D + \beta_D \cdot [\text{GdnHCl}]) \cdot \exp\left(\frac{\Delta G_{D-N}^{H_2O} + m_{D-N} \cdot [\text{GdnHCl}]}{-R \cdot T}\right)}{1 + \exp\left(\frac{\Delta G_{D-N}^{H_2O} + m_{D-N} \cdot [\text{GdnHCl}]}{-R \cdot T}\right)}$$

(eq. 12)

Values of the observed fluorescence and the related guanidine hydrochloride concentration are fitted into this equation. Hereby the values of $\Delta G_{D-N}^{H_2O}$ and m_{D-N} with standard errors are obtained. From equation 11 it obvious that at the point where 50% of the protein is denatured the free energy is given by:

$$\Delta G_{D-N}^{H_2O} = m_{D-N} \cdot [\text{GdnHCl}]_{50\%} \quad \text{(eq. 13)}$$

At the point where the reaction is at equilibrium the free energy is zero, $\Delta G_{\text{D-N}}^{[\text{GdnHCl}]} = 0$. Resultantly, $[\text{GdnHCl}]_{50\%}$ is given by:

$$[\text{GdnHC}]_{50\%} = \frac{\Delta G_{\text{D-N}}^{\text{H}_2\text{O}}}{m_{\text{D-N}}} \quad \text{(eq. 14)}$$

At any given guanidine hydrochloride concentration the free energy is given by inserting equation 11 into equation 13:

$$\Delta G_{\text{D-N}}^{\text{GdnHCl}} = m_{\text{D-N}} \cdot ([\text{GdnHCl}]_{50\%} - [\text{GdnHCl}]) \quad \text{(eq. 15)}$$

Calculation of the difference in the free energy upon mutation can be done in three different ways:[218]

1. $\Delta\Delta G_{\text{D-N}}^{[\text{GdnHCl}]_{50\%}} = \langle m_{\text{D-N}} \rangle \cdot \Delta[\text{GdnHCl}]_{50\%}$ (eq. 16)

   where $\langle m_{\text{D-N}} \rangle$ is the average value of *m* for wild-type and mutant.

2. When an average value of $m_{\text{D-N}}$ is neglect $\Delta\Delta G_{\text{D-N}}$ can also be calculated by

   $$\Delta\Delta G_{\text{D-N}}^{\text{H}_2\text{O}} = \Delta G_{\text{D-N}}^{\text{H}_2\text{O}} - \Delta G'^{\text{H}_2\text{O}}_{\text{D-N}} \quad \text{(eq. 17)}$$

   where $\Delta G_{\text{D-N}}^{\text{H}_2\text{O}}$ and $\Delta G'^{\text{H}_2\text{O}}_{\text{D-N}}$ are the free energies of unfolding in water for wild type and mutant, respectively.

3. Furthermore, it can be calculated at any given guanidine hydrochloride concentration by

   $$\Delta\Delta G_{\text{D-N}}^{[\text{GdnHCl}]} = m_{\text{D-N}} \cdot ([\text{GdnHCl}]_{50\%} - [\text{GdnHCl}]) - m'_{\text{D-N}} \cdot ([\text{GdnHCl}]'_{50\%} - [\text{GdnHCl}]')$$

   (eq.18)

   where [GdnHCl]' and *m*' represents values for the mutant and [GdnHCl] and *m* values for the wild type protein.

Fersht and co-workers have examined the three methods and found that the method 3) has the smallest error.[216] Their results of repeating experiments afforded values of

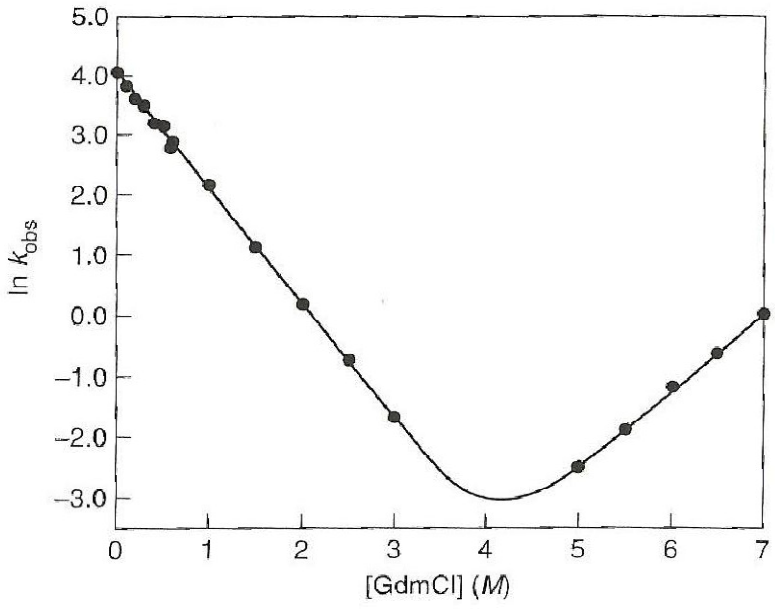
$[GdnHCl]_{50\%}$ which was reproducible within 0.02 M However, the values of *m* and therefore also $\Delta G_{D-N}^{H_2O}$ were significantly larger. Even a small error in *m* leads to a large error in $\Delta G_{D-N}^{H_2O}$ because of the long extrapolation from the transition region (approximately 4-5 M guanidine hydrochloride) to zero molar guanidine hydrochloride. The values of $\Delta\Delta G_{D-N}^{[GdnHCl]_{50\%}}$ were reproducible within 0.1 kcal/mol versus the values of $\Delta\Delta G_{D-N}^{H_2O}$ that were only reproducible within 0.5 kcal/mol.

The calculated values of $\Delta\Delta G_{D-N}^{[GdnHCl]_{50\%}}$ can be used for calculating the Φ-values because $\Delta\Delta G_{D-N}^{[GdnHCl]_{50\%}} = -\Delta\Delta G_{D-N}^{[GdnHCl]_{50\%}}$

5.3.3 Kinetic experiments

Kinetic measurements of guanidine hydrochloride denaturation involve a stop-flow fluorescence spectrofluorometer. It mixes the protein-buffer solution with the guanidine hydrochloride solution very rapidly and has a very small "dead time" before measurements are made. This small dead time is crucial as α-helices form within a few hundred nanoseconds, β-strands within a few microseconds and short loops within 10^{-6} seconds.[198] The data obtained by the stopped-flow experiments are fluorescence versus time, which follows exponential curves. The equations of these exponential curves and the related rate constants are obtained by computational data fitting. The rate constants versus guanidine hydrochloride concentration are plotted in a so called chevron plot as illustrated in figure 16.

Figure 16. Chevron plot for wild type CI2.[198]



The right side of the chevron plot arises from unfolding experiments and the left side of the plot from refolding experiments. For unfolding experiments the protein is kept in a buffer solutions and each data point is a result of mixing the protein-buffer solution with a solution containing a given guanidine hydrochloride concentration. For refolding experiments the protein is kept in a solution with a high guanidine hydrochloride concentration and is then mixed in different ratios with a buffer solution to provide each data point on the curve. The chevron plots are the basis for further computational data fitting.[216,218,220]

Fitting the unfolding and folding kinetics cannot be obtained using the same equations as for the thermodynamic experiments. Proline-isomerization has to be taken into account. Despite the fact that proline in the *trans* conformation is highly favoured there is still 2-20% of the *cis* conformation in the denatured state. In native CI2 all prolines are *trans* and the conversion from *cis* to *trans* is relatively slow and thereby also the folding phase is slow. In the kinetics this accounts for approximately 20-30% of the amplitude.[198]

The unfolding data is fitted to a single-exponential equation as the unfolding in monophasic:

$$F(t) = A_0 \cdot \left(1 - e^{-k_D \cdot t}\right) - m \cdot t + C \quad \text{(eq. 19)}$$

where *F(t)* is the fluorescence at time *t*, A_0 is the amplitude, k_D is the rate constant, *m* is the slope of the drift and *C* is an offset. The folding kinetics is fitted into a multiple-exponential equation due to proline-isomerization giving a triphasic refolding reaction:

$$F(t) = A_1 \cdot \left(e^{-k_1 \cdot t}\right) + A_2 \cdot \left(e^{-k_2 \cdot t}\right) - m \cdot t + C \quad \text{(eq. 20)}$$

where A_1 and A_2 are the amplitudes. Plots of the natural logarithm of the rate constant versus the guanidine hydrochloride concentration (before the transition state is reached) are linear:

$$\ln k_{\mathrm{D}} = \ln k_{\mathrm{D}}^{\mathrm{H_2O}} + m_{k\mathrm{D}} \cdot [\mathrm{GdnHCl}] \quad \text{(eq. 21)}$$

where k_{D} is the rate constant of unfolding at a given guanidine hydrochloride concentration and $k_{\mathrm{D}}^{\mathrm{H_2O}}$ is the rate constant of unfolding in water and $m_{k\mathrm{D}}$ is the slope.

Similarly, the plots of the natural logarithm of the rate constant against the guanidine hydrochloride concentration (after the transition state is reached) are also linear:

$$\ln k_{\mathrm{N}} = \ln k_{\mathrm{N}}^{\mathrm{H_2O}} + m_{K\mathrm{N}} \cdot [\mathrm{GdnHCl}] \quad \text{(eq. 22)}$$

where k_{N} is the rate constant of folding at a given guanidine hydrochloride concentration and $k_{\mathrm{N}}^{\mathrm{H_2O}}$ is the rate constant of folding in water and $m_{K\mathrm{N}}$ is the slope. The complete kinetics used for data fitting is:

$$\ln k = \ln k_D + \ln k_N = \ln\left(k_{\mathrm{D}}^{\mathrm{H_2O}} \cdot \exp\left(m_{k\mathrm{D}} \cdot [\mathrm{GdnHCl}]\right) + k_{\mathrm{N}}^{\mathrm{H_2O}} \exp\left(m_{K\mathrm{N}} \cdot [\mathrm{GdnHCl}]\right)\right)$$

(eq. 23)

In addition, $[\mathrm{GdnHCl}]_{50\%}$ can also be determined:

$$[\mathrm{GdnHCl}]_{50\%} = \frac{\ln\left(k_{\mathrm{N}}^{\mathrm{H_2O}} / k_{\mathrm{D}}^{\mathrm{H_2O}}\right)}{m_{k\mathrm{D}} - m_{k\mathrm{N}}} \quad \text{(eq. 24)}$$

As previously described the free energy at a particular denaturant concentration is defined as $\Delta G_{\mathrm{D-N}}^{[\mathrm{GdnHCl}]} = -RT \ln K_{\mathrm{D\text{-}N}}$ and therefore the difference in energy of the transition state of the unfolding relative to the folded state between wild-type and mutant, $\Delta\Delta G_{\ddagger\text{-N}}$, is defined as:

$$\Delta\Delta G_{\ddagger\text{-N}} = -RT \ln\left(\frac{k_{\mathrm{D}}}{k'_{\mathrm{D}}}\right) \quad \text{(eq. 25)}$$

where k_{D} and k'_{D} are the rate constants for the wild-type and the mutant, respectively. Similar the difference in energy of the transition state of the folding relative to the folded state between wild-type and mutant, $\Delta\Delta G_{\ddagger\text{-D}}$, is calculated:

$$\Delta\Delta G_{\ddagger\text{-D}} = -RT \ln\left(\frac{k_{\mathrm{N}}}{k'_{\mathrm{N}}}\right) \quad \text{(eq. 26)}$$

where k_N and k'_N are the rate constants for the wild type protein and the mutant, respectively. This is used for calculation of Φ. The most accurate value of $\Delta\Delta G_{\ddagger\text{-D}}$ is that calculated at $[GdnHCl]_{50\%}$ as this minimizes errors due to limited extrapolation.

5.4 Concluding remarks

Recently, there has been an increased interest in a better understanding of protein folding due to the focus on diseases arising from protein misfolding. Since the 1950's several different folding mechanisms have been proposed. However, no complete understanding has yet been reached, and the research within this field is ongoing. The protein engineering method is one way of studying protein folding. It involves mutation of a well-known protein with a simple folding mechanism (e.g. CI2). The difference in stability upon mutation and the difference in energy of the transition state upon mutation are measured by guanidine hydrochloride denaturation. Different methods can be utilized to monitor the denaturation, e.g. by fluorescence. An extensive study on several CI2 mutants has been conducted, but there is still gaps to fill out. In particular there is a need to mutate residues within the α-helical region to form more stable mutants. This would make it clearer if formation of the α-helix initiates the folding of CI2.

5.5 Aim of the project

Despite the extensive experiments performed with CI2 there are still many unsolved issues in terms of how to interpret the observed folding rates upon mutation.

This project is a continuation of the investigation on how a change in the backbone of CI2 will affect the ability of the protein to fold. Fersht and co-workers have made almost 100 mutants of CI2, however not all mutants afforded the expected results.[218] In a few cases it was not possible to calculate Φ-values, namely mutations of residue 18 and 22. Interestingly, the mutations of these residues to alanine resulted in no change in stability of the protein. The results were unexpected and therefore a different choice of mutant should be examined in order to form more stable proteins. Furthermore, most of the mutants made by Fersht and co-workers destabilized the protein. It would also be of great interest to make mutations of other residues, which would increase stability of the protein. Hopefully, it will complement the ester study previously performed in the group[228] (and also unpublished results). Stabilization of the protein is attempted by introducing a helical inducing amino acid. What is of particular interest for this project is the evaluation of the source of several negative Φ-value towards the C-terminus of the α-helix. This will complement the work by Fersht and co-workers, where the predictions of the computational work could not be tested well enough by side-chain mutations.[218]

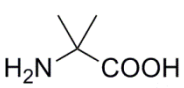
5.6 Results and discussion

5.6.1 Mutation choice

The choice of mutation site and the nature of the mutant are of great importance. It must not produce new interactions or structural rearrangements to the folded protein since that can alter the folding pathway. However, at the same time it must change the stability of the protein in order for Φ-values to be determined.[198,216]

The chosen amino acid to, hopefully, increase the stability of CI2 is α-aminoisobutyric acid, Aib (figure 17).

Figure 17. Structure of Aib.



Aib is a natural non-protein amino acid found in membrane-channel-forming peptides of microbial origin.[229] It possesses high α–helical propensity, even higher than that of alanine, which has already been mentioned as being the most α-helical favouring amino acid among the 20 protein amino acids. Aib should decrease the chain entropy of the unfolded state due to the presence of an additional methyl group. This will reduce the allowed conformational space and the torsion angles of the backbone will be more restricted. Aib does not affect either the secondary or the tertiary structure when it replaces alanine.[229]

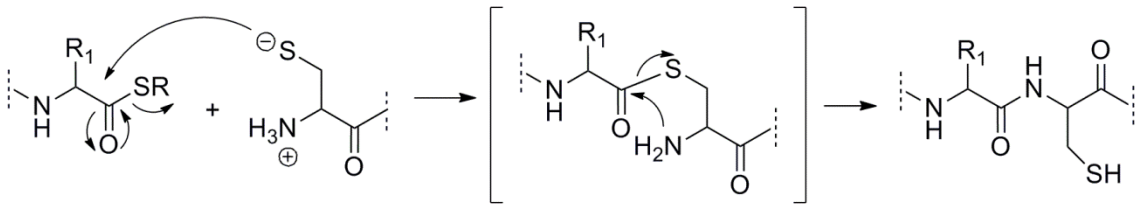
Besides mutation with Aib of residues 18 and 22 also residues 15 and 16 is examined. In wild type CI2 residues 15 and 16 are alanine, and in the native state they already posses some helical structure. It is expected that mutation to Aib will result in high Φ-values.

5.6.2 Protein synthesis

The CI2 mutants are made by chemical synthesis using standard procedures. If each protein consisting of 64 amino acids were to be synthesised in one linear peptide synthesis, the overall yield would most likely be low due to the many coupling reactions. Even if each coupling yield is high, e.g. in 99.5% yield this will result in overall yield of only 72.6%. If the each coupling is just slightly lower, e.g. in 99.0% yield the overall yield drops to 52.5%. An overall yield of merely 3.8% is achieved if each coupling is in 95.0% yield. Therefore, it is crucial to get each coupling in the highest possible yield. However, even if all couplings are quantitative there is a big risk of aggregation due to the large size of the peptide/protein.

5.6.3 Chemical ligation

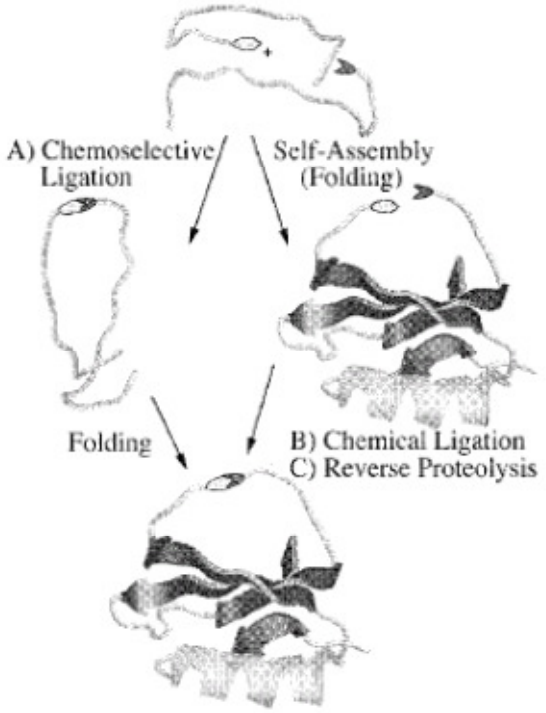
Due to the problems concerning coupling yields and aggregation it is practically not possible to synthesise peptides larger than 50 amino acids. Therefore chemical ligations of peptide fragments have been widely studied. One of the most commonly used methods is native chemical ligation developed by Dawson *et al* in 1994.[230,231] It involves the solution phase reaction between two unprotected peptides in a guanidine hydrochloride buffer. One peptide contains a C-terminal thioester and the other a N-terminal cysteine residue. This reaction affords a natural peptide backbone structure as illustrated in scheme 100.



Scheme 100. Native chemical ligation.

Interestingly, the requirement of the N-terminal cysteine is not always necessary. If the two segments can fold to bring the C- and N-termini in close proximity then the ligation of the two pieces is via a regular peptide coupling. This can be performed with any given amino acid residue and it does not require the usual strong coupling reagents. It is performed in an aqueous buffer, which induces folding. This technology is termed conformationally or folding assisted ligation. An illustrative description of both native chemical and folding assisted ligations is provided in figure 18.[232]

Figure 18. A) Native chemical ligation and B) folding assisted ligation.[232]



It has been shown that fragments corresponding to CI2(1-40) and CI2(41-64) can self-associate to form a stable protein with a crystal and solution structure very close to that of intact CI2.[233] Therefore, it was envisioned that two fragments of CI2 would be able to ligate by folding assisted ligation. In the Dawson group fragments of CI2, namely CI2(1-39) and CI2(40-64), have previously been examined with mutation of residue 39 from threonine to aspartic acid. It was found that folding assisted ligation enhanced the

reaction rate of the ligation from 48 hours (by native chemical ligation) to only 30 minutes by folding assisted ligation.[232] The mutation of residue 39 to aspartic acid does not affect the folding/unfolding reaction.[218] Therefore, it was chosen for this project to synthesize similar segments; CI2(1-39) as the C-terminal thioesters and CI2(40-64) as C-terminal acids.

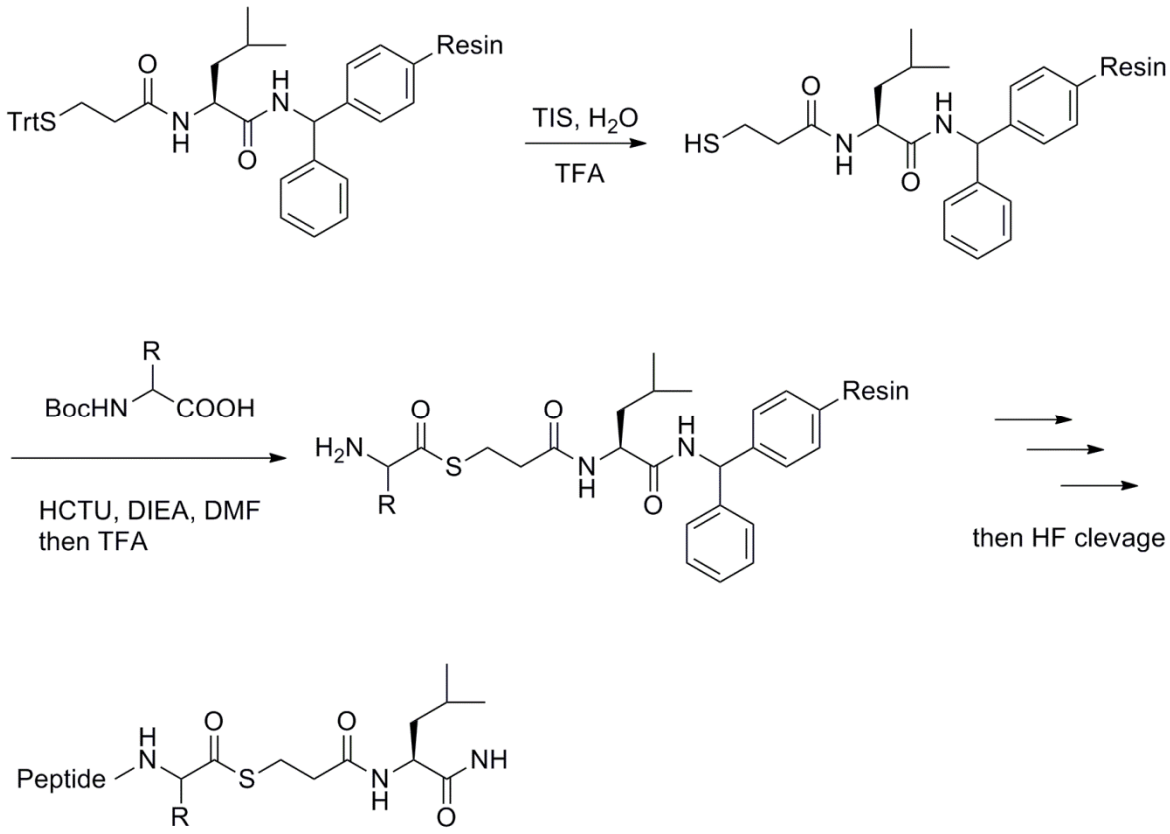
5.6.4 Solid phase peptide synthesis

The two CI2 segments, CI2(1-39)COSR and CI2(40-64)COOH, can be made by solid phase peptide synthesis (SPPS), which was developed by R. B. Merrifield in 1963.[234]

SPPS can be performed by two strategies, which involve different temporary protection groups, *tert*-butyloxycarbonyl (Boc) or 9-fluorenylmethoxycarbonyl (Fmoc). Boc is acid labile, whereas Fmoc is base labile. As a consequence of this, the two strategies involve entirely different reagents for removal of the temporary protection group as well as the permanent protection groups. Additionally, different methods are employed when the peptide is cleaved from the solid support. In general, the Fmoc-strategy is more commonly used compared to the Boc-strategy due to the fact that Boc-strategy requires liquid HF for cleaving of peptide from the solid support. This is a potential hazard and is preferably avoided. However, Fmoc-strategy is generally incompatible with peptide thioesters, as the base required for deprotection of temporary protection groups also cleaves the thioester. Due to the great importance of ligations, particularly native chemical ligation, Fmoc compatible methods for synthesis of peptide thioesters has recently been reported. [235-238] Nevertheless, in the Dawson group peptide thioesters made by the Boc-strategy is well established and was the chosen procedure for this project. Instead, CI2(40-64)COOH is easily be made by the Fmoc-strategy.

5.6.4.1 CI2(1-40)COSR by Boc-strategy

CI2(1-39) were made as C-terminal thioesters on the TAMPAL resin using manual Boc-strategy as shown in scheme 101. It involves standard SPPS conditions; Removal of temporary protection groups with a TFA (the trityl-group on the TAMPAL resin was initially removed by a TFA-cocktail with 2.5% TIS and 2.5% water), pre-activation of amino acids with HCTU and DIEA and DMF as solvent. After the final Boc-deprotection TFA the peptide was cleaved from the resin (as well as deprotection of permanent protection groups) by treatment with anhydrous liquid HF.

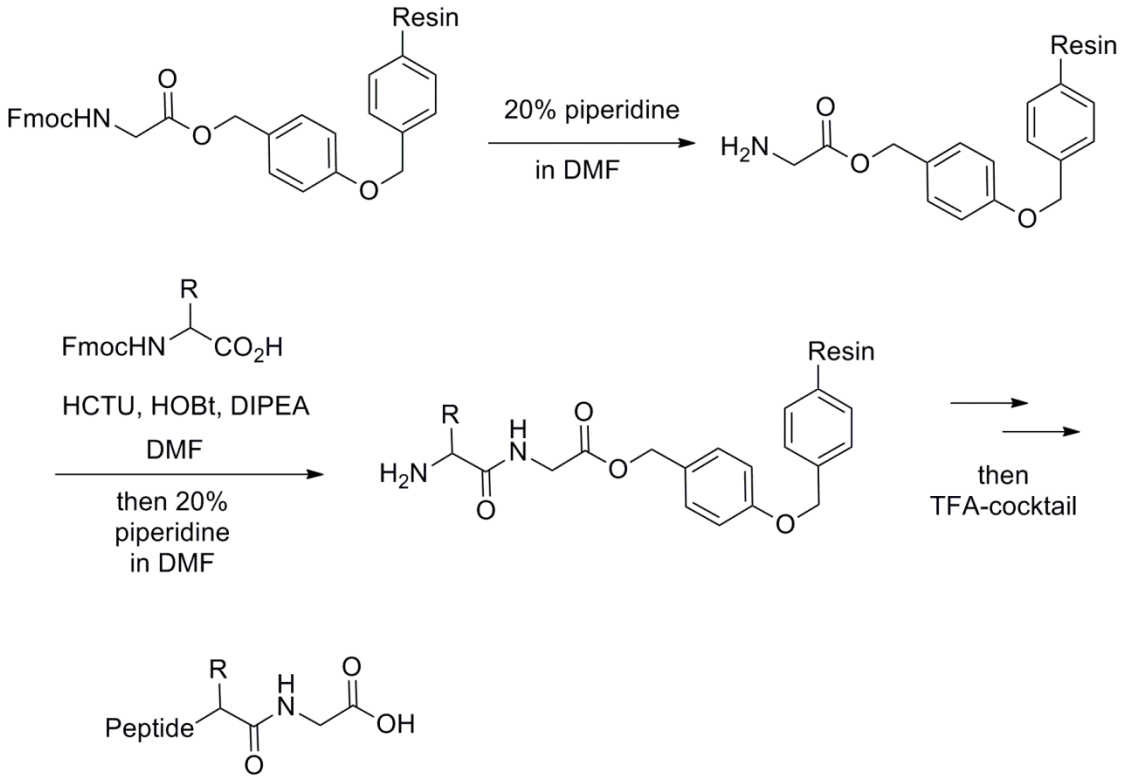


Scheme 101. Boc-strategy

Amino acids were used in excess (5.5-fold) to allow couplings to go faster (10-15 minutes) and thereby also limiting unwanted side reactions. However, the first coupling is the most difficult and a coupling time of 40 minutes was employed. HCTU is not able to couple the sterically hindered amino acid Aib. Therefore the stronger (and more expensive) coupling reagent HATU was used with a 10-fold excess of the amino acid and a coupling time of 30 minutes as well as a deprotection time of 2 minutes.

5.6.4.2 CI2(41-64)COOR by Fmoc-strategy

CI2(40-64) was made as C-terminal acids on Wang resin using automated Fmoc strategy. The Wang resin with glycine precoupled is commercially available and the coupling scheme for Fmoc SPPS of the peptide building block is shown in scheme 102. As for the Boc-strategy, this Fmoc-strategy also involves standard reaction conditions; firstly, deprotection of the temporary protection group by piperidine in DMF, then the amino acid was coupled in presence of HCTU, HOBt and DIPEA. Removal from resin was achieved by treatment of a suitable TFA-cocktail (5% DCM, 2.5% TIS and 2.5% water).



Scheme 102. Fmoc-strategy.

HOBt is an additive that prevents side reactions and racemisation.[239] It is acidic enough to provide some competition in the removal of the proton from the carbon of the activated species. Though, it is not acidic enough to deprotonate a free amine. The first amino acid was coupled manually as it requires pre-activation with DIC and DMAP. Amino acids were used in a 10-fold excess with coupling and deprotection times of 20 minutes each.

5.6.5 Folding studies

The CI2 mutants synthesised and analysed by both thermodynamic and kinetic experiments are:

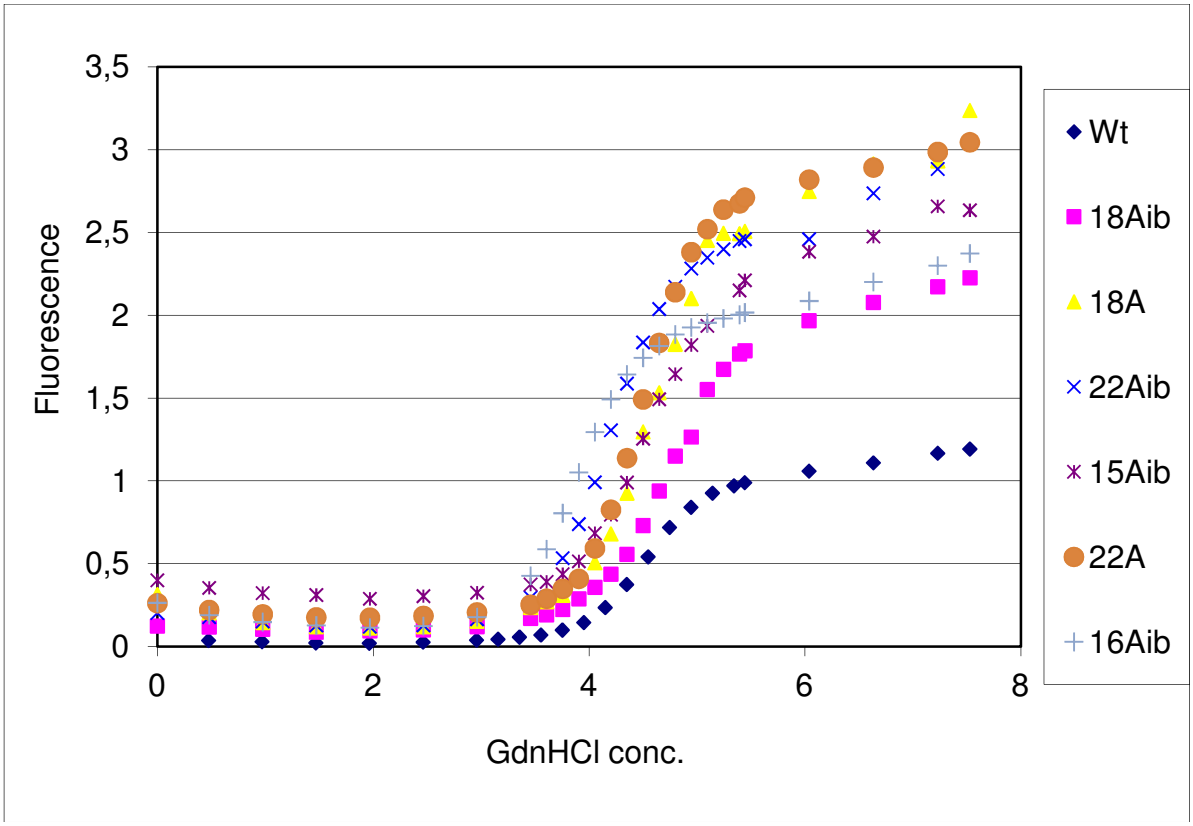
- CI2-A15Aib
- CI2-A16Aib
- CI2-K18Aib
- CI2-K18A
- CI2-Q22Aib
- CI2-Q22A
- CI2-wt

CI2-K18A and CI2-Q22A are used as control experiments. Results of these should be identical to those reported by Fersht and co-workers.[218]

5.6.5.1 Thermodynamics

The results of the guanidine hydrochloride denaturation experiments monitored by fluorescence are summarized in figure 19.

Figure 19. Guanidine hydrochloride denaturation (unfitted data) of CI2 mutants and wild type.



Computational data fitting of the obtained fluorescence versus guanidine hydrochloride results was conducted by Associate Professor Evan Powers and is summarized in figure 20 and table 22.

Figure 20. Fitted data from guanidine hydrochloride denaturation of CI2 mutants and wild type.

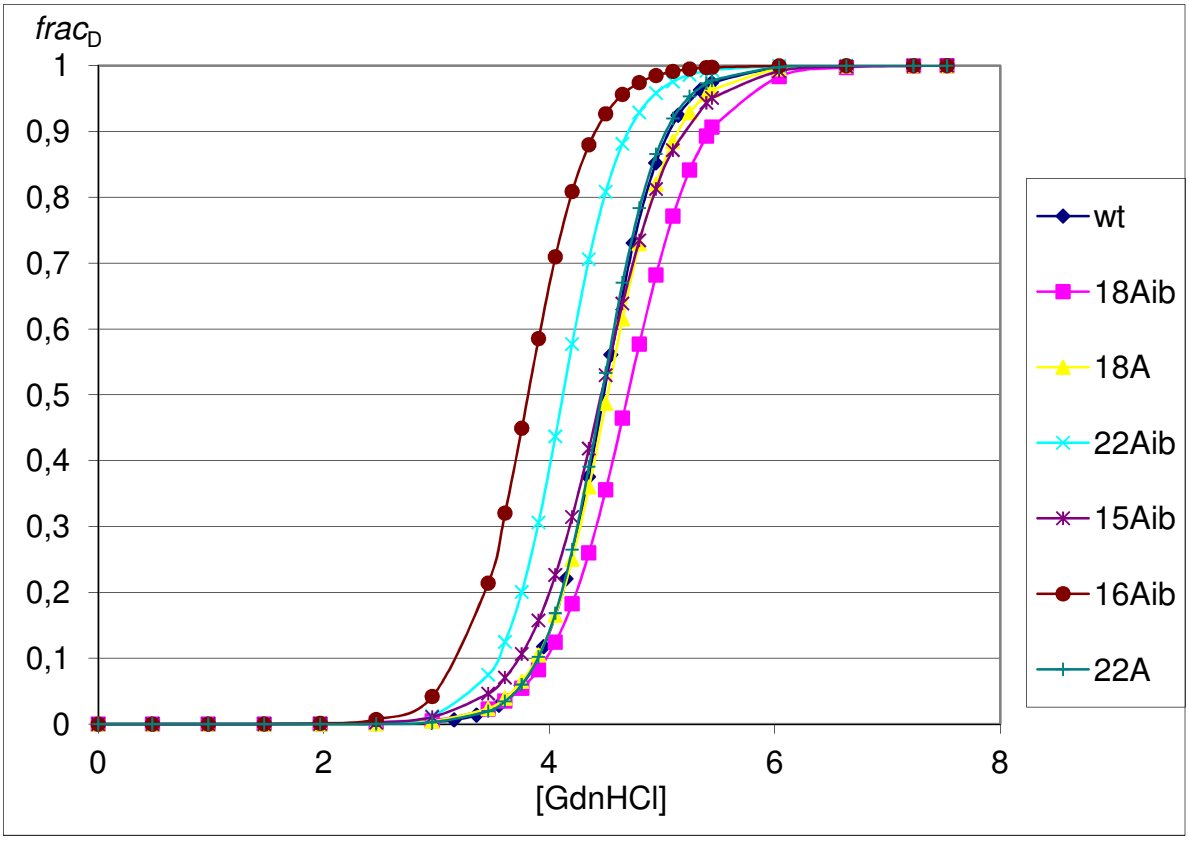


Table 22. Calculated values from thermodynamic guanidine hydrochloride denaturation

Protein	$[GdnHCl]_{50\%}$	$[GdnHCl]_{50\%}$ Reported	*m*	*m* Reported	$\Delta G_{D\text{-}N}^{[GdnHCl]}$	$\Delta G_{D\text{-}N}^{[GdnHCl]}$ Reported	$\Delta G_{D\text{-}N}^{[GdnHCl]_{50\%}}$
CI2-wt	**4.48**	4.00	**2.24**	1.90	**10.06**	7.60	**-**
CI2-K18A	**4.52**	4.11	**2.08**	1.61	**9.38**	6.61	**-0.06**
CI2-K18Aib	**4.70**	-	**1.80**	-	**8.45**	-	**-0.41**
CI2-Q22A	**4.47**	3.99	**2.29**	1.77	**10.23**	7.06	**0.03**
CI2-Q22Aib	**4.12**	-	**2.24**	-	**9.24**	-	**0.70**
CI2-A15Aib	**4.46**	-	**1.79**	-	**7.97**	-	**0.04**
CI2-A16Aib	**3.81**	-	**2.18**	-	**8.31**	-	**1.30**

(<m>) = 2.09, which in the literature is 1.94.[218]

Unfortunately, the results are not in accordance with literature values. As seen in the table the three known proteins (CI2-wt, K18A and Q22A) all afforded significantly higher values of $[GdnHCl]_{50\%}$ and $\Delta G_{D\text{-}N}^{[GdnHCl]}$, which implies that the synthesized proteins are significantly more stable compared to the proteins made by Fersht and co-workers. This is not possible as they are identical proteins and an error in the measurements must have taken place.

5.6.5.2 Kinetics

The kinetic measurement of guanidine hydrochloride denaturation shows fluorescence versus time. The obtained data was fitted by computational methods by Associate Professor Evan Powers. Two examples of folding of CI2-A15Aib at approximately 3.2 M guanidine hydrochloride are provided in figure 21 and 22. It is clear from figure 22 that the procedure is very sensitive and data fitting can become very complicated and often result in unusable data.

Figure 21. An example of folding of CI2 suitable for data analysis.

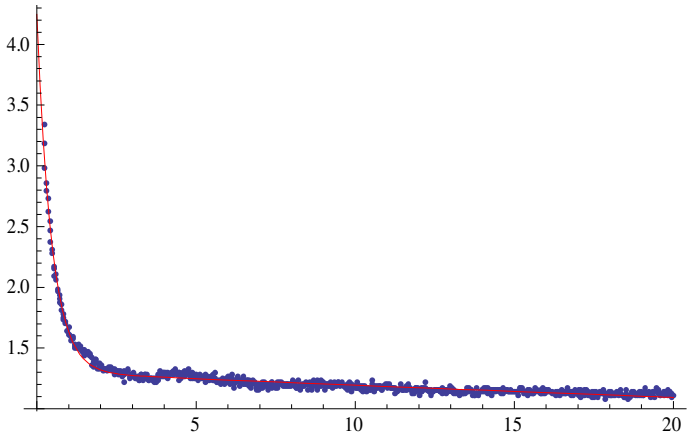
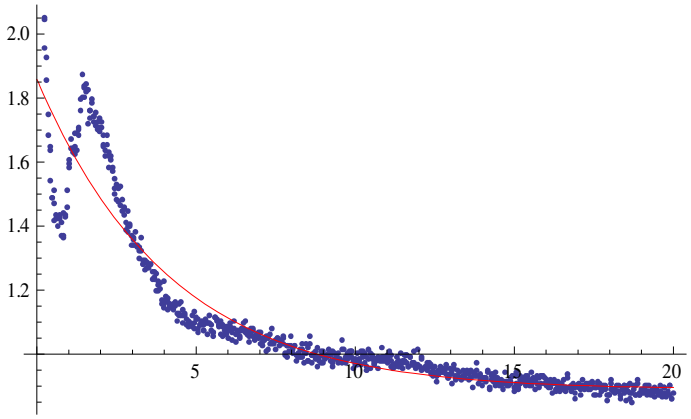


Figure 22. An example the influence of proline-isomerization in folding of CI2.



Results of unfolding of the CI2-A15Aib are illustrated in figure 23 and 24, with the latter affording best results of data fitting.

Figure 23. An example of unfolding of CI2 not suitable for data analysis.

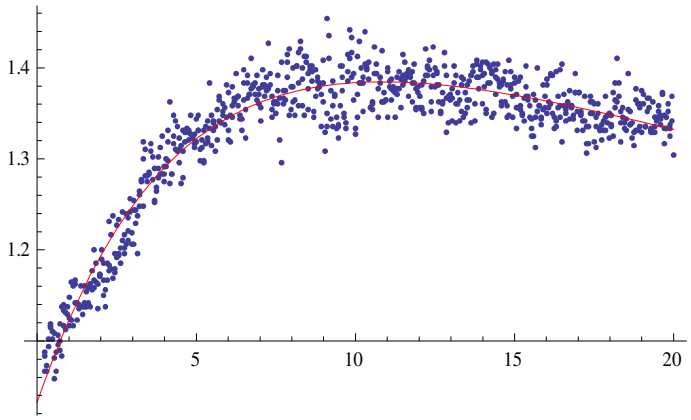
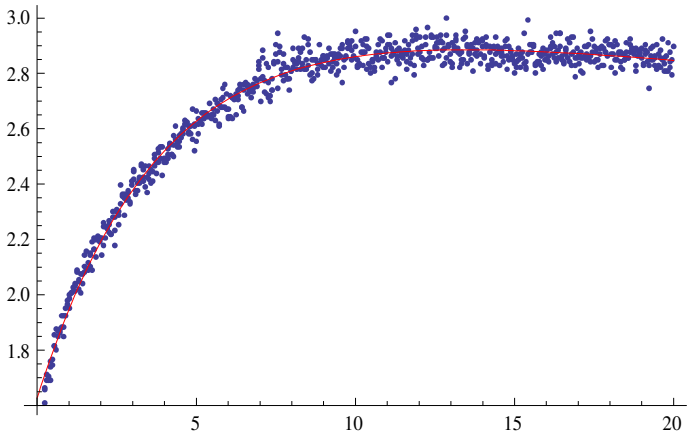
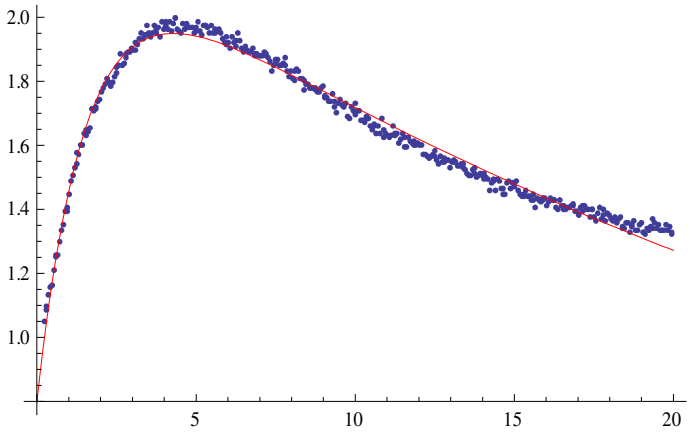


Figure 24. An example of unfolding of CI2 suitable for data analysis.



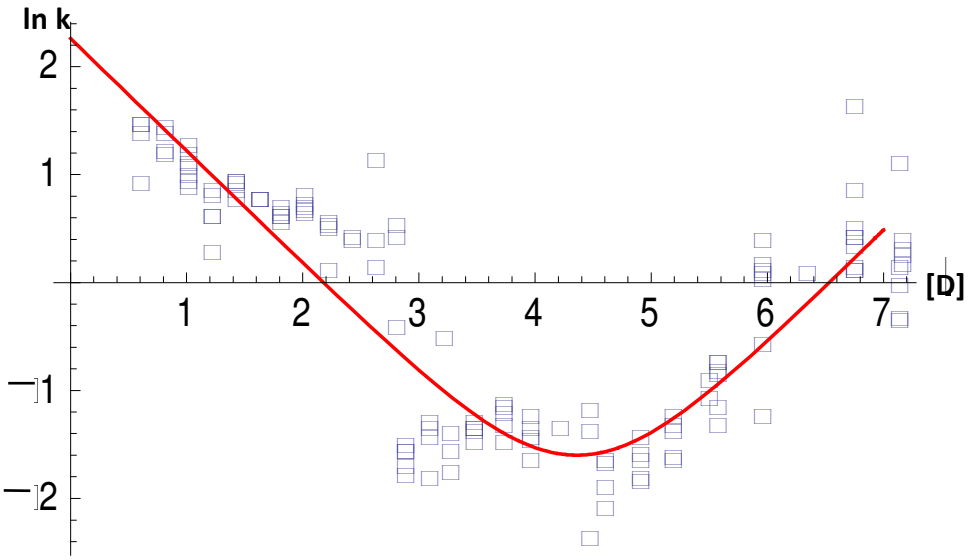
Interestingly, sometimes unexpected results were obtained as illustrated in figure 25 (folding of CI2-K18A at approximately 3.0 M guanidine hydrochloride). Despite the good fit, the reaction constant is off, and results are not usable for calculation of the Gibbs free energy.

Figure 25. Unexpected results of unfolding of CI2.



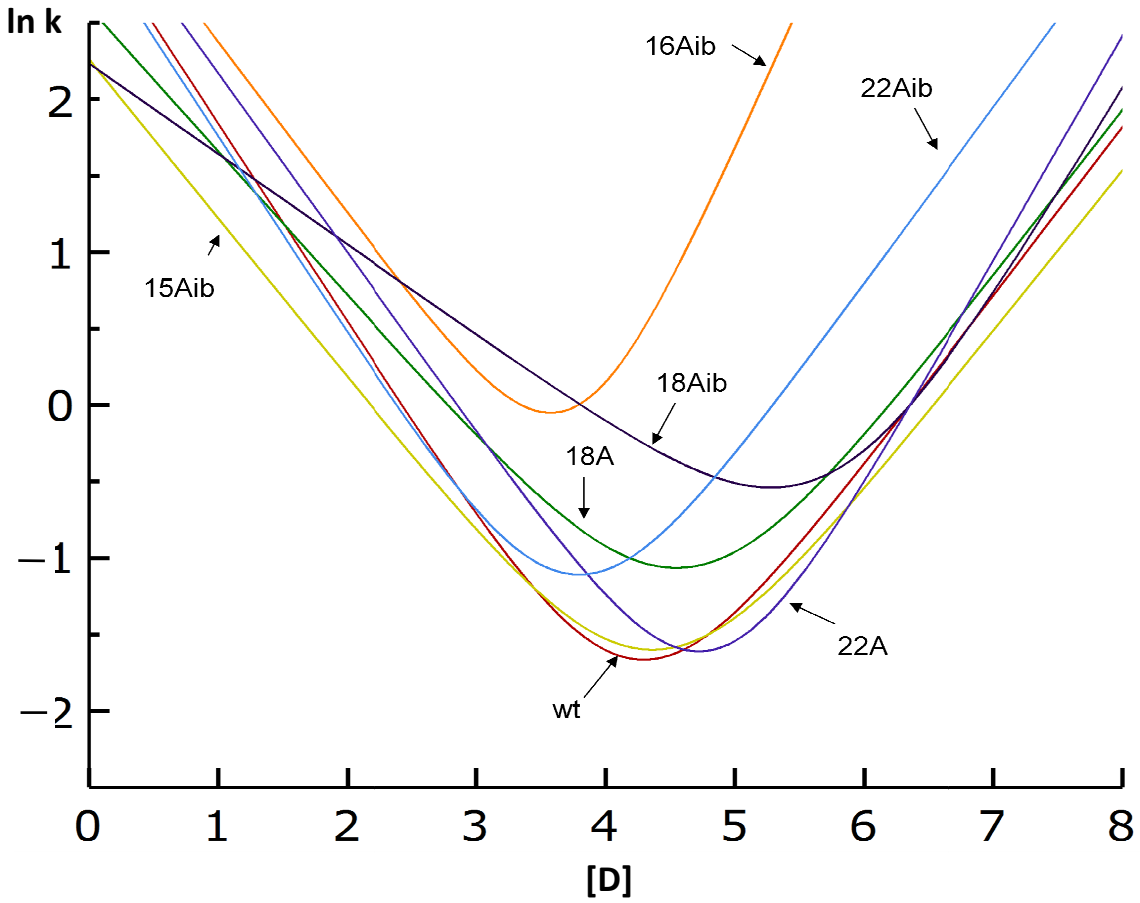
For each mutant the same experiment is repeated five times as the technique is very sensitive and the smallest air bobble in the solutions can affect the results tremendously. Each experiment affords a curve as those above and the rate constants are calculated and plotted versus guanidine hydrochloride concentration in the chevron plot. This is exemplified with CI2-A15Aib in figure 26, which clearly emphasizes the need for repeated experiments as some rate constants are far off. It also makes data analysis rather tedious as the poor results has to be manually removed.

Figure 26. Chevron plot for CI2-A15Aib.



Combinations of all chevron plots of the synthesized proteins are provided in figure 27.

Figure 27. All chevron plots.



The curves in the chevron plots were expected to have the same slope for all CI2 mutants. Different slopes imply different folding mechanisms, which is not the case for CI2 mutants. The curves to the right of wild type CI2 indicate less stable proteins and to the left more stable proteins. Interestingly, all mutants fold faster than wild type CI2 as they are placed higher in the diagram (have higher rate constants). Data of rate constants and *m*-values are listed in table 23 and the literature values are listed in table 24.

Table 23. Calculated values of ln *k* and *m*.

Entry	Protein	$\ln k_N^{H_2O}$	m_N	$\ln k_D^{H_2O}$	m_D
1	CI2-wt	3.14 ± 0.16	-1.30 ± 0.06	-7.03 ± 0.51	1.11 ± 0.08
2	CI2-K18A	2.72 ± 0.10	-1.02 ± 0.04	-6.81 ± 0.50	1.10 ± 0.08
3	CI2-K18Aib	2.23 ± 0.10	-0.59 ± 0.04	-9.16 ± 1.28	1.40 ± 0.19
4	CI2-Q22A	3.34 ± 0.14	-1.17 ± 0.05	-9.39 ± 0.68	1.48 ± 0.11
5	CI2-Q22Aib	3.05 ± 0.14	-1.29 ± 0.06	-6.11 ± 0.35	1.15 ± 0.06
6	CI2-A15Aib	2.26 ± 0.14	-1.04 ± 0.07	-6.89 ± 0.65	1.05 ± 0.10
7	CI2-A16Aib	3.49 ± 0.28	-1.12 ± 0.13	-7.81 ± 1.62	1.90 ± 0.35

Table 24. Literature values of ln *k* and *m*.

Entry	Protein	$\ln k_N^{H_2O}$	m_N	$\ln k_D^{H_2O}$	m_D
1	CI2-wt	4.03 ± 0.04	-1.82 ± 0.12	-9.04 ± 0.07	1.31 ± 0.01
2	CI2-K18A	3.90 ± 0.01	-1.77 ± 0.03	-8.89 ± 0.13	1.31 ± 0.02
4	CI2-Q22A	4.24 ± 0.02	-2.15 ± 0.05	-8.98 ± 0.68	1.32 ± 0.01

Unfortunately, also the kinetic results are not in accordance with the literature values. The errors are significantly larger than those for the literature values. This indicates that there is an error in the spetrofluorometer. Air bobbles was a recurrent problem during the experiments, which might be held accountable.

In table 25 the $[GdnHCl]_{50\%}$-values for both kinetic and thermodynamic experiments as well as the literature values are listed. Not only are the results not agreeing with literature values, they are also in disagreement with each other.

Table 25. Values of $[GdnHCl]_{50\%}$

Entry	Protein	$[GdnHCl]_{50\%}$ kinetics	$[GdnHCl]_{50\%}$ thermodynamics	$[GdnHCl]_{50\%}$ literature
1	CI2-wt	4.22	4.48	4.00
2	CI2-K18A	4.50	4.52	4.11
3	CI2-K18Aib	5.72	4.70	-
4	CI2-Q22A	4.80	4.47	3.99
5	CI2-Q22Aib	3.75	4.12	-
6	CI2-A15Aib	4.38	4.46	-
7	CI2-A16Aib	3.74	3.81	-

Values of the change in stability of the transition state upon mutation, $\Delta\Delta G_{\ddagger\text{-D}}$, from the kinetic experiments as well as literature values are listed in table 26. Also values of change in stability of the protein upon mutation are provided. Obviously, neither terms of stability are in agreement with literature values as a consequence of the difference in $[GdnHCl]_{50\%}$-values.

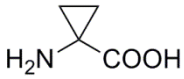
Table 26. Gibbs free energies.

Entry	Protein	$\Delta\Delta G_{D-N}^{[GdnHCl]_{50\%}}$ kinetics	$\Delta\Delta G_{D-N}^{[GdnHCl]_{50\%}}$ thermodynamics	$\Delta\Delta G_{D-N}^{[GdnHCl]_{50\%}}$ literature	$\Delta\Delta G_{‡\text{-}D}$ kinetics	$\Delta\Delta G_{‡\text{-}D}$ literature
1	CI2-wt	1.38	**-**	-	-	
2	CI2-K18A	1.10	**-0.06**	-0.15	-0.25	0.08
3	CI2-K18Aib	0.68	**-0.41**	-	-0.54	-
4	CI2-Q22A	1.35	**0.03**	-0.11	0.12	-0.12
5	CI2-Q22Aib	1.06	**0.70**	-	-0.05	-
6	CI2-A15Aib	1.36	**0.04**	-	-0.52	-
7	CI2-A16Aib	0.41	**1.30**	-	0.21	-

As a consequence of the inaccuracy of the obtained results from both thermodynamic and kinetic experiments it will not make any sense to calculate the Φ-values. Once that spectrofluorometers are repaired new measurements can be conducted.

Despite the unreliable denaturation results, it seems that the majority of the mutants are less stable compared to wild type. Therefore, also mutation of residue 14, 15, 16, 18 and 22 into 1-aminocyclopropanecarboxylic acid (Acpc, figure 28) was examined as a α-helical inducing residue. However, due to time restraints only CI2(1-39)Acpc-mutation thioesters still on resin were prepared. These need to be cleaved from the resin and ligated with CI2(40-64) before used for guanidine denaturation experiments.

Figure 28. 1-aminocyclopropanecarboxylic acid.



5.7 Conclusion

Several CI2 mutants have successfully been prepared by folding assisted ligation of peptide segments prepared by solid phase peptide synthesis. The mutations sites are all within the α-helical region. Residues were mutated into alanine and Aib, which both posses a highly α-helical propensity. The mutants were prepared by folding assisted ligation of two segments, CI2(1-40)thioesters and CI2(41-64). The peptides have been synthesized by either Boc- or Fmoc-strategies. The proteins were used for transition state analysis. Guanidine hydrochloride denaturation was monitored by fluorescence both in terms of thermodynamics and kinetics. Unfortunately, the obtained results of reference compounds revealed errors in the apparatus as the values were not in accordance with the literature. Nevertheless, there is an indication of the majority of the mutants being less stable compared to the wild type protein. Therefore, also thioester segments with mutations into 1-aminocyclopropanecarboxylic acid were prepared. The cyclopropyl group might enhance the rate of formation of the α-helix and form more stable proteins. Due to time restraints the full proteins were not prepared and analysed.

6 Summary

Many biologically active molecules contain nitrogen either as primary/secondary/tertiary amines or as part of a heterocycle (aromatic or non-aromatic). Resultantly, the field of C-N bond formation receives great attention, and herein three different projects in this area have been carried out. All are concerned with C-N formation by the borrowing hydrogen methodology, *i.e.* N-alkylation of amines with alcohols or amines catalyzed by iridium or ruthenium. The theme was "green chemistry" in terms of developing new and more environmentally benign reactions for the use in pharmaceutical research. Focus has been on employment of catalysis, reducing the amount of waste (hence increasing the E-factor), pursue high atom economy and avoid the use of toxic/hazardous reagents.

The first project was based on the serendipitous discovery that a primary amine in the presence of the commercially available $[Cp^*IrCl_2]_2$ catalyst afforded a secondary amine. Primary alifatic and benzylic amines were tolerated in the reaction, and the products were obtained in good to high yields. The method did not require use of a solvent or an additive and isolation of the products were achieved by simple distillations directly from the reaction mixtures. Unfortunately, the procedure afforded significantly lower yields if two different amines were reacted to afford unsymmetrical secondary amines. Additionally, intramolecular cyclization of primary amines failed completely.

The second project dealt with the synthesis of piperazines primarily from diamines and diols catalyzed by $[Cp^*IrCl_2]_2$. Generally, it was difficult to prepare piperazine itself as the reaction required at least one substituent on one of the starting materials. C- and N-substituted piperazines were obtained in high yields. The simplest starting material for synthesis of piperazine is ethanolamine, though this led to disappointing results due to polymerization. Also, aryl amines were not suitable as starting materials as by-products were obtained in large quantities. The use of ruthenium catalysis ($RuCl_3$ in combination with either PPh_3 or xantphos) in the same reaction did not reveal any product formation. It was investigated if other sources of nitrogen could be employed. Interestingly, ammonium tetrafluoroborate was efficient, however, a morpholine derivative was formed as the major product instead of the expected piperazine.

The third project involved formation of indoles from anilines and vicinal diols. Ruthenium-phosphine catalytic systems ($RuCl_3$ in combination with either PPh_3 or xantphos) were

employed and the products were obtained in good yields. The reactions were performed under neat conditions and addition of additives was avoided.

The fourth project was conducted as a part of an external stay at the Scripps Research Institute, San Diego, California, USA. It focused on protein folding of a well-known protein, CI2. The goal was to synthesize more stable mutants compared to the wild type protein and to measure the difference in energy of the transition state upon mutation. This was performed by guanidine denaturation monitored by fluorescence. These results would be a part of a larger study on how to understand protein folding better. Unfortunately, due to errors in the spectrofluorometer the obtained results were unreliable.

7 Experimental work performed at DTU

7.1 Materials and methods

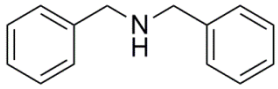
All chemicals were purchased from commercial sources and used without further purification. Solvents as well as starting materials were used without distillation or further removal of water. Solvents used for chromatography were of HPLC grade. Thin layer chromatography was performed on aluminum plates coated with silica gel 60. Visualization was done by UV or by dipping in a solution of cerium(IV)sulfate (2.5 g) and ammonium molybdate (6.25 g) in 10% sulfuric acid (250 mL) or ninhydrin (10g) in ethanol (300mL) followed by charring with a heatgun. Column chromatography was performed on silica gel (220-440 mesh). GC were conducted on a Shimadzu GC2010 instrument equipped with an Equity™ 1 column (15 m × 0.1 mm, 0.1 µm film). GC-MS was performed on a Shimadzu GCMS-QP5000 instrument equipped with Equity-1 capillary column (30 m × 0.25 mm, 0.25 µm film) or Shimadzu GCMS-QP2010S instrument equipped with an Equity-5 column (30 m x 0.25 mm x 0.25 µm film). GC-yields were obtained using *m*-xylene as internal standard. High resolution mass spectra were recorded at the Department of Physics and Chemistry, University of Southern Denmark or at the Department of Systems Biology, Technical University of Denmark. ^{1}H and ^{13}C NMR spectra were obtained on a Varian Mercury 300 instrument at 300 MHz and 75 MHz, respectively. The chemical shifts are reported in ppm relative to the residual deuterated solvent.[240] Melting points are uncorrected.

7.2 Secondary amines

General methods for the synthesis of secondary amines:

A primary amine (16 mmol) and $[Cp^*IrCl_2]_2$ (0.08 mmol) were added to a 5-mL screw-top heavy-walled vial. The vial was flushed with argon, sealed and placed in an aluminum block and heated to 170 °C for 18 hours (unless otherwise stated). After cooling to room temperature DCM (2 mL) was added and the resulting solution was transferred to a round bottomed flask and DCM was removed on a rotary evaporator. The desired product was isolated by distillation *in vacuo*.

Dibenzylamine



Yield = 70%

Bp: 143–145 °C/10 mbar (Lit.[241] 113–114 °C/0.13 mbar).

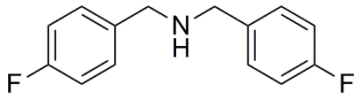
^{1}H NMR ($CDCl_3$): δ 7.25–7.35 (m, 10 H), 3.78 (s, 4 H), 1.56 (s, 1 H).

^{13}C NMR ($CDCl_3$): δ 140.3, 128.3, 128.1, 126.8, 53.1.

NMR data is in accordance with literature values.[96]

MS (EI, 70 eV): *m/z* = 197 [M].

Bis(4-fluorobenzyl)amine



Yield = 83%

Bp: 130–132 °C/3 mbar.

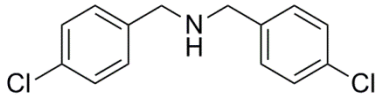
^{1}H NMR ($CDCl_3$): δ 7.30–6.97 (m, 8 H), 3.74 (s, 4 H).

^{13}C NMR ($CDCl_3$): δ 161.9 (d, *J* = 245 Hz), 135.9 (d, *J* = 3.2 Hz), 129.6 (d, *J* = 7.8 Hz), 115.1 (d, *J* = 21 Hz), 52.3.

NMR data is in accordance with literature values.[242]

MS (EI, 70 eV): *m/z* = 233 [M].

Bis(4-chlorobenzyl)amine



Yield = 80%

Bp: 177–181 °C/3 mbar (Lit.[243] 230 °C/20 mbar).

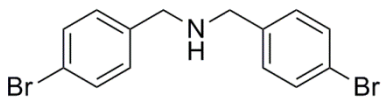
^{1}H NMR ($CDCl_3$): δ 7.30–7.26 (m, 8 H), 3.73 (s, 4 H),1.56 (s, 1 H).

^{13}C NMR ($CDCl_3$): δ 138.5, 132.6, 129.4, 128.4, 52.2.

NMR data is in accordance with literature values.[96]

MS (EI, 70 eV): *m/z* = 266 [M].

Bis(4-bromobenzyl)amine



Yield = 76%

Mp: 46–48 °C (Lit.[244] 48–49 °C);

Bp 145–147 °C/0.04 mbar.

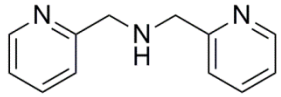
^{1}H NMR ($CDCl_3$): δ 7.44 (d, *J* = 8.2 Hz, 4 H), 7.20 (d, *J* = 8.2 Hz, 4 H), 3.72 (s, 4 H).

^{13}C NMR ($CDCl_3$): δ 139.1, 131.4, 129.8, 120.7, 52.3.

NMR data is in accordance with literature values.[244]

MS (EI, 70 eV): *m/z* = 355 [M].

Bis[(2-pyridyl)methyl]amine



Yield = 79%

Bp: 107–110 °C/0.09 mbar (Lit.[245] 130–135 °C/0.13 mbar).

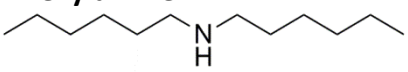
^{1}H NMR ($CDCl_3$): δ 8.50–8.48 (m, 2 H), 7.56 (td, *J* = 1.7 and 7.7 Hz, 2 H), 7.28 (d, *J* = 7.8 Hz, 2 H), 7.10–7.06 (m, 2H), 3.91 (s, 4 H), 2.52 (s, 1 H).

^{13}C NMR ($CDCl_3$): δ 159.5, 149.1, 136.3, 122.1, 121.8, 54.6.

NMR data is in accordance with literature values.[245]

MS (EI, 70 eV): *m/z* = 200 [M + H].

Dihexylamine



Yield = 72% (72 h reaction time)

Bp: 85–86 °C/3 mbar (Lit.[246] 75 °C/1.3 mbar).

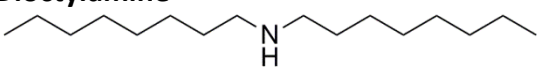
^{1}H NMR ($CDCl_3$): δ 2.59 (t, *J* = 7.6 Hz, 4 H), 1.48 (m,4 H), 1.29 (m, 12 H), 0.89 (t, *J* = 6.5 Hz, 6 H).

^{13}C NMR ($CDCl_3$): δ 50.1, 31.7, 30.1, 27.0, 22.5, 13.9.

NMR data is in accordance with literature values.[247]

MS (EI, 70 eV): *m/z* = 185 [M].

Dioctylamine



Yield = 72% (72 h reaction time)

Bp: 139–141 °C/3 mbar (Lit.[248] 145–155 °C/4 mbar).

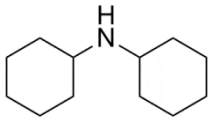
^{1}H NMR ($CDCl_3$): δ 2.50 (t, *J* = 7.3 Hz, 4 H), 1.39 (m,4 H), 1.20 (br s, 20 H), 0.79 (t, *J* = 6.7 Hz, 6 H).

^{13}C NMR ($CDCl_3$): δ 50.1, 31.7, 30.1, 29.4, 29.2, 27.3, 22.5, 13.9.

NMR data is in accordance with literature values.[247]

MS (EI, 70 eV): *m/z* = 241 [M].

Dicyclohexylamine



Yield = 73% (68 h reaction time)

Bp: 93–95 °C/5 mbar (Lit.[114] 79–81 °C/0.5 mbar).

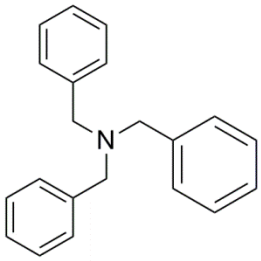
^{1}H NMR ($CDCl_3$): δ 2.55 (m, 2 H), 1.91–1.81 (m, 4 H), 1.76–1.67 (m, 4 H), 1.65–1.56 (m, 2 H), 1.32–0.95 (m, 10 H).

^{13}C NMR ($CDCl_3$): δ 52.9, 34.2, 26.1, 25.2.

NMR data is in accordance with literature values.[76]

MS (EI, 70 eV): *m/z* = 181 [M].

Tribenzylamine



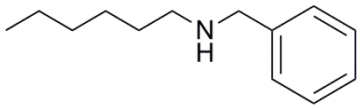
Yield = max. 37% (72h reaction time, not reproducible)

^{1}H NMR ($CDCl_3$): δ 7.43-7.20 (m, 15 H), 3.56 (s, 6H).

^{13}C NMR ($CDCl_3$): δ 139.6, 128.7, 128.2, 126.8, 57.9.

NMR data is in accordance with literature values.[36]

N-hexylbenzylamine



Hexylamine (1.9 mmol), benzylamine (1.9 mmol) and $[Cp^*IrCl_2]_2$ (0.019 mmol) were added to a 5-mL screw-top heavy-walled vial. The vial was flushed with argon, sealed and placed in an aluminum block and heated to 170 °C for 18h. After cooling to room temperature CH_2Cl_2 (2 mL) the reaction mixture was purified by flash column chromatography (EtOAc:heptane 4:1).

Yield = 40%

^{1}H NMR ($CDCl_3$): δ. 7.32-7.22 (m, 5H), 3.78 (s, 2H), 2.62 (t, 2H), 1.55-1.46 (m, 2H), 1.36-1.25 (m, 7H), 0.88 (t, 2H).

^{13}C NMR ($CDCl_3$): δ 140.8, 128.6, 128.3, 127.1, 54.3, 49.8, 32.0, 30.3, 27.3, 22.9, 14.3.

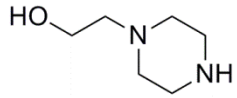
NMR data is in accordance with literature values.[249]

7.3 Piperazines

General procedure for iridium catalyzed reactions:

To a 5 mL screw-top vial were added all reactants and catalyst, additive and solvent (when mentioned). The vial was flushed with argon, sealed, placed in an aluminum block and heated to the indicated temperature for overnight reaction (unless otherwise stated). After cooling to room temperature the reaction mixture was transferred to a round bottom flask and the solvent was removed *in vacuo*. The residue was further purified by column chromatography (heptane/EtOAc or MeOH/CH_2Cl_2 mixtures) unless otherwise stated.

1-(2-Hydroxyethyl)piperazine



Ethanolamine (8.3 mmol), $[Cp^*IrCl_2]_2$ (2 x 10^{-5} mmol) and $NaHCO_3$ (0.1 mmol) at 140 ^oC.

Yield = 30% (water as solvent, 4 days reaction time).

Yield = 23% (neat, 2 days reaction time).

Yield = 14% (neat, 6 days reaction time).

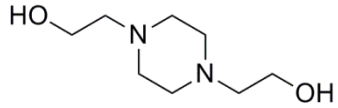
^1H NMR (CD_3OD): 3.68 (t, 2H), 2.92 (t, 4H), 2.56-2.51 (m, 6H).

^{13}C NMR (CD_3OD): 61.7, 59.7, 54.4, 45.8.

NMR data is identical to that of compound purchased from Sigma-Aldrich.

MS: *m/z* 130 [M^+].

1,4-Bis(2-hydroxyethyl)piperazine



Ethanolamine (8.3 mmol), $[Cp^*IrCl_2]_2$ (2 x 10^{-5} mmol) and $NaHCO_3$ (0.1 mmol) at 140 ^oC.

Yield = 27% (water as solvent, 4 days reaction time).

Yield = 25% (neat, 2 days reaction time).

Yield = 20% (neat, 6 days reaction time).

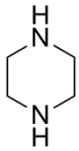
^1H NMR (CD_3OD): 3.70 (t, 4H, *J* = 5.9 Hz), 2.73 (bs, 8H), 2.65 (t, 4H, *J* = 5.9 Hz).

^{13}C NMR (CD_3OD): 60.9, 59.4, 53.6;

NMR data is identical to that of compound purchased from Sigma-Aldrich.

HRMS calcd for $C_8H_{19}N_2O_2$ $[M+H]^+$ *m/z* 174.1447, found *m/z* 175.1448.

Piperazine



2-(2-Aminoethylamino)ethanol (5.0 mmol), $[Cp^*IrCl_2]_2$ (0.0125 mmol), $NaHCO_3$ (0.1 mmol) and water (3 mL) at 140 ^oC. After cooling to room temperature the reaction mixture was transferred to a distillation unit and solvent and product were distilled off together. To this was added 6M HCl and solvent removed in vacuo affording the product as a hydrochloride salt.

Yield = 79% yield.

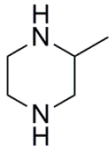
^1H NMR ($CDCl_3$): 2.78 (s, 8H, C*H*$_2$).

^{13}C NMR ($CDCl_3$): 47.2.

NMR data is in accordance with literature values.[250]

MS: *m/z* 86 [M^+].

(±)-2-methylpiperazine



Diamine (4 mmol), diol (4 mmol), $[Cp^*IrCl_2]_2$, (0.02 mmol), $NaHCO_3$ (0.02 mmol) and solvent (1 mL) at 140 ^oC. After cooling to room temperature the reaction mixture was transferred to a distillation unit and solvent and product were distilled off together. To this was added 6M HCl and solvent removed in vacuo affording the product as a hydrochloride salt.

From 1,2-diaminopropane and ethyleneglycol:

Yield = 69% (water as solvent)

Yield = 69% (toluene as solvent)

From ethylenediamine and 1,2-propanediol:

Yield = 92% (water as solvent)

Yield = 72% (tolene as solvent)

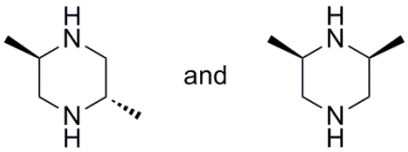
^1H NMR (D_2O): 2.71-2.62 (m, 3H), 2.55-2.33 (m, 3H), 2.10-2.02 (dd, 1H, *J* = 10.5, 12.5 Hz, C*H*H), 0.78 (d, 3H, *J* = 6.4 Hz, C*H*$_3$).

^{13}C NMR (D_2O): 51.4, 50.2, 45.0, 44.0, 18.5.

NMR data is in accordance with literature values.[251]

MS: *m/z* 100 [M^+].

(±)-*trans*-2,5- and (±)-*cis*-2,6-Dimethylpiperazine



1,2-Diaminopropane (4 mmol), 1,2-propanediol (4 mmol), $[Cp^*IrCl_2]_2$, (0.02 mmol), $NaHCO_3$ (0.02 mmol) and solvent (1 mL) at 140 ^oC. After cooling to room temperature the reaction mixture was transferred to a distillation unit and solvent and product were distilled off together. To this was added 6M HCl and solvent removed in vacuo affording the products as a hydrochloride salts.

Yield = 90% (water as solvent), 1.5:1 ratio.

Yield = 73% (toluene as solvent), 1:1 ratio.

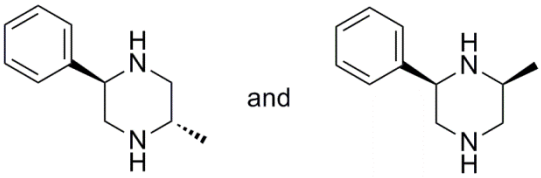
^1H NMR ($CDCl_3$): 2.87-2.82 (dd, 2H, *J*=11.8, 2.8 Hz, HNC$H_{eq}H_{ax}$), 2.65-2.56 (m, 2H, C$H_{ax}CH_3$), 2.35-2.28 (major) (dd, 1H, *J* = 10.3, 11.7 Hz, HNCH$_{eq}H_{ax}$), 2.21-2.14 (minor) (m, 1H, *J* = 10.3, 12.2 Hz, HNCH$_{eq}H_{ax}$), 0.94-0.92 (minor) (d, 3H, *J* = 6.3 Hz, CH_3), 0.93-0.91 (major) (d, 3H, *J* = 6.3 Hz, CH_3).

^{13}C NMR ($CDCl_3$): 54.0 (major), 52.8 (minor), 51.9 (minor), 50.8 (major), 19.7 (minor), 19.5 (major).

NMR data is identical to that of compound purchased from Sigma-Aldrich.

MS: *m/z* 114 [M^+].

(±)-*trans*-1-phenyl-5-methylpiperazine and (±)-*cis*-1-phenyl-6-methylpiperazine



1,2-Diaminopropane (4 mmol), 1-phenyl-1,2-ethanediol (4 mmol), $[Cp^*IrCl_2]_2$, (0.02 mmol), $NaHCO_3$ (0.02 mmol) and solvent (1 mL) at 160 ^oC. After cooling to room temperature the reaction mixture was transferred to a distillation unit and products were isolated under high vacuum.

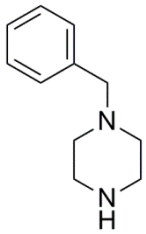
Yield = 60% (water as solvent), 3:1 ratio.

Yield = 70% (toluene as solvent), 5:4 ratio.

^{1} NMR ($CDCl_3$): 7.22-7.41 (m, 5H, Ar-H), 3.80 (minor) (dd, 1H, *J* = 2.9, 10.4 Hz), 3.72 (major) (dd, 1H, *J* = 2.9, 10.4 Hz), 3.08 (major) (2xdd, 2H, PhCHCH_{eq} and CH_3CHCH_{eq}), 2.98 (minor) (2xdd, 2H, PhCHCH_{eq} and CH_3CHCH_{eq}), 2.89 (m, 1H, CH_3C*H*), 2.78 (major) (dd, 1H, *J* = 10.3, 11.9 Hz, PHCHCH_{ax}), 2.62 (minor) (dd, 1H, *J* = 10.3, 12.0 Hz, PHCHCH_{ax}), 2.55 (major) (dd, 1H, *J* = 10.4, 11.1 Hz, CH_3CHCH_{ax}), 2.42 (minor) (dd, 1H, *J* = 10.5, 12.3 Hz, CH_3CHCH_{ax}), 1.01 (d, 3H, *J* = 6.2 Hz, CH_3).

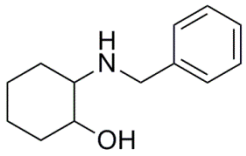
^{13}C NMR ($CDCl_3$): 142.6 (minor), 142.4 (major), 128.3 (major and minor), 127.3 (major), 127.3 (minor), 126.9 (minor), 126.6 (major), 61.2 (major), 54.7 (major), 54.4 (major), 52.4 (minor), 53.5 (minor), 53.0 (minor), 52.6 (minor), 50.7 (major), 20.0 (minor), 19.8 (major).
HRMS calcd. for $C_{11}H_{17}N_2$ $[M+H]^+$ *m/z* 177.1386, found *m/z* 177.1407

N-Benzylpiperazine



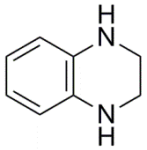
N-benzylethylenediamine (2 mmol), ethyleneglycol (2 mmol), $[Cp^*IrCl_2]_2$, (0.01 mmol), $NaHCO_3$ (0.1 mmol) and toluene (1 mL) at 160^{o}C.
Yield = 63% yield
^{1}H NMR ($CDCl_3$): 7.32-7.31 (m, 5H, Ar-H), 3.49 (s, 2H, Ph-CH_2), 2.89 (t, 4H *J* = 4.5 Hz), 2.42 (br s, 4H), 2.33 (s, NH).
^{13}C NMR ($CDCl_3$): 137.9, 129.2, 128.1, 126.9, 63.6, 54.3, 45.9.
NMR data is in accordance with literature values.[252]
MS: *m/z* 176 $[M^+]$.

2-(Benzylamino)cyclohexanol



Benzylamine (2.2 mmol), 1,2-cyclohexanediol (2 mmol), $[Cp^*IrCl_2]_2$, (0.01 mmol), $NaHCO_3$ (0.1 mmol) and toluene (1 mL) at 110 ^{o}C.
Yield = 54%.
^{1}H NMR ($CDCl_3$): 7.30-7.32 (m, 4H, Ar-H), 7.22-7.28 (m, 1H, Ar-H), 3.93 (d, 1H, *J* = 12.9 Hz, PhC*H*), 3.67 (d, 1H JA, *J* = 12.9 Hz, PhC*H*), 3.14-3.22 (m, 1H, HOC*H*), 2.28 (ddd, 1H, *J* = 2.2, 7.1, 9.2 Hz, HOCHC*H*NH), 1.96-2.02 (m, 1H), 2.10-2.18 (m, 1H), 1.68-1.72 (m, 2H), 1.14-1.29 (m, 4H, 3xCH, NH), 0.91-1.04 (m, 1H).
^{13}C NMR ($CDCl_3$): 140.4, 128.3, 128.0, 126.9, 73.6, 63.0, 50.7, 33.3, 30.4, 25.0, 24.3.
NMR data is in accordance with literature values.[253]
MS: *m/z* 205 $[M^+]$.

1,2,3,4-Tetrahydroquinoxaline



1,2-Diaminobenzene (2 mmol), ethyleneglycol (3 mmol), $[Cp^*IrCl_2]_2$ (0.01 mmol), $NaHCO_3$ (0.1 mmol) and mesitylene (1 mL) at 180 ^oC.

Yield = 34%.

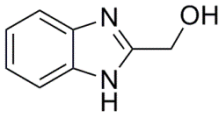
^1H NMR ($CDCl_3$): 6.61-6.56 (m, 2H), 6.53-6.48 (m, 2H) 3.42 (s, 4H).

^{13}C NMR ($CDCl_3$): 133.6, 118.7, 114.7, 41.3.

NMR data is in accordance with literature values.[254]

MS: *m/z* 134 [M^+].

2-benzimidazolemethanol



1,2-Diaminobenzene (2 mmol), ethyleneglycol (3 mmol), $[Cp^*IrCl_2]_2$ (0.01 mmol), $NaHCO_3$ (0.1 mmol) and mesitylene (1 mL) at 180 ^oC.

Yield = aprox. 30% (trace of impurities present).

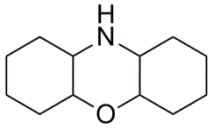
^1H NMR (CD_3OD): 7.56-7.50 (m, 2H, Ar-H), 7.23-7.17 (m, 2H, Ar-H), 4.89 (s, NH), 4.83 (s, 2H, HOCH_2).

^{13}C NMR (CD_3OD): 156.2, 123.4, 59.0.

NMR data is in accordance with literature values.[255]

MS: *m/z* 148 [M^+].

Dodecahydro-1*H*-phenoxazine



1,2-Cyclohexanediol (2.0 mmol) and NH_4BF_4 (1.0 mmol), $[Cp^*IrCl_2]_2$ (0.025 mmol), TFA (0.1 mmol) and mesitylene (1mL) at 180 ^oC.

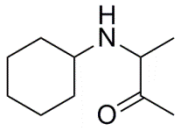
Yield = 63%.

^1H NMR ($CDCl_3$): 3.19-3.11 (m, 2H), 2.63 (s, NH), 2.54-2.47 (m, 2H), 1.90-1.83 (m, 2H), 1.74-1.66 (m, 6H), 1.35-1.23 (m, 8H); δ_C (75 MHz, $CDCl_3$).

^{13}C NMR ($CDCl_3$): 80.9, 60.7, 31.0, 30.8, 24.5, 24.5.

HRMS calcd. for $C_{12}H_{22}NO$ $[M+H]^+$ *m/z* 196.1696, found *m/z* 196.1698.

3-(Cyclohexylamine)butan-2-one



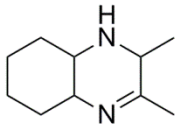
Acetoin (1 mmol), cyclohexylamine (1mmol) and toluene (1 mL) at 60 ^{o}C for 1 hour. Yield was not determined as the compound was only used for mechanistic purposes.

^{1}H NMR ($C_6D_5CD_3$): 3.12 (q, 1H, *J* = 6.9 Hz, CH_3CHNH), 2.25-2.17 (m, 1H, $NHCHCH_2$), 1.80 (s, 3H, $COCH_3$), 1.69-1.53 (m, 6H), 1.12-1.00 (m, 4H), 0.93 (d, 3H, *J* = 6.9 Hz, $NHCHCH_3$).

^{13}C NMR ($C_6D_5CD_3$): 210.2, 60.6, 55.4, 34.3, 33.9, 26.5, 25.7, 25.2, 25.1, 18.7.

HRMS calcd. for $C_{10}H_{20}NO$ $[M+H]^+$ *m/z* 170.1539, found *m/z* 170.1539.

2,3-Dimethyl-5,6,7,8,9,10-hexahydroquinoxaline



Acetoin (1 mmol), 1,2-diaminocyclohexane (1 mmol) and toluene-d_8 (1 mL) at 60 ^{o}C for 4 hours. Compound not purified.

^{13}C NMR ($CD_3C_6D_5$): 158.0, 59.2, 33.8, 25.7, 22.3.

NMR data is in accordance with literature values.[256]

MS: *m/z* 164 $[M^+]$.

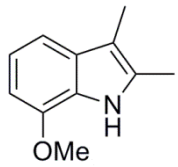
General procedure for ruthenium catalyzed reactions:

To a 5 mL screw-top vial were added $RuCl_3 \cdot xH_2O$, (0.02 mmol), PPh_3 (0.06 mmol) or xantphos (0.03 mmol), diamine (2 mmol), diol (2 mmol) and solvent (1 mL). The vial was flushed with argon, sealed and placed in an aluminum block and heated to the indicated temperature for overnight reaction. After cooling to room temperature samples were taken for GC-MS analysis. No work-up.

7.4 Indoles

To a 5 mL screw-top vial were added the aniline (2 mmol), the diol (2 mmol), $RuCl_3 \bullet xH_2O$ (0.02 mmol) and triphenylphosphine (0.06 mmol) or xantphos (0.03 mmol). The vial was flushed with argon, sealed and placed in an aluminum block and heated to 110 °C for 1 hour and then heated to 170 °C for 24 hours (unless otherwise stated). After cooling to room temperature the reaction mixture was transferred to a round bottom flask and solvent was removed *in vacuo*. The residue was further purified by column chromatography (heptane/EtOAc, DCM/MeOH or DCM/heptanes mixtures).

7-Methoxy-2,3-dimethylindole



Yield = 41% (PPh_3).

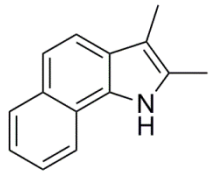
Yield = 34% (xantphos).

^{1}H NMR ($CDCl_3$): 2.20 (s, 3H), 2.33 (s, 3H), 3.93 (s, 3H), 6.58 (d, 1H, *J* = 7.5 Hz), 6.99 (t, 1H, *J* = 7.8 Hz), 7.90 (d, 1H, *J* = 7.8 Hz), 7.90 (br s, 1H).

^{13}C NMR ($CDCl_3$): 8.7, 11.5, 55.3, 101.2, 107.5, 111.0, 119.3, 125.2, 130.2, 130.7, 145.3.

HRMS: *m*/*z*: calcd for $C_{11}H_{12}NO$: 174.0924 $[M\text{-}H]^-$; found: 174.0928.

2,3-Dimethyl-1*H*-benzo[*g*]indole



Yield = 60% (PPh_3),

Yield = 72% (xantphos, 2 days reaction time)

Mp: 149 °C (lit.[257] m.p. 153-155 °C).

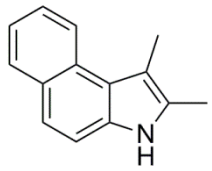
^{1}H NMR ($CDCl_3$): 2.29 (s, 3H), 2.45 (s, 3H), 7.36 (ddd, 1H, *J* = 7.8, 6.9, 1.2 Hz), 7.45 (ddd, 1H, *J* = 8.2, 6.9, 1.2 Hz), 7.47 (d, 1H, *J* = 8.2 Hz), 7.61 (d, 1H, *J* = 8.4 Hz), 7.87-7.95 (m, 2H), 8.40 (br s, 1H).

^{13}C NMR ($CDCl_3$): 8.6, 11.6, 118.6, 119.0, 119.7, 121.2, 123.0, 125.1, 128.8, 129.9.

NMR data is in accordance with literature values.[258]

MS: *m*/*z* 195 [M].

1,2-Dimethyl-3*H*-benzo[*e*]indole



Yield = 58% (PPh_3,2 days reaction time).

Yield = 63% (xantphos,2 days reaction time).

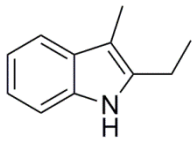
Mp: 118-121 °C (lit.[259] m.p. 131 °C).

^{1}H NMR ($CDCl_3$): 2.39 (s, 3H), 2.61 (s, 3H), 7.37 (d, 1H, *J* = 8.7 Hz), 7.37 (ddd, 1H, *J* = 8.4, 6.9, 1.5 Hz), 7.50(d, 1H, *J* = 8.7 Hz), 7.51 (ddd, 1H, *J* = 8.4, 6.9, 1.5 Hz), 7.90 (d, 1H, *J* = 8.8 Hz), 7.94 (br s, 1H), 8.49 (d, 1H, *J* = 8.4 Hz).

^{13}C NMR ($CDCl_3$): 11.5, 12.4, 109.7 (2C), 112.2, 121.8, 122.5, 131.1, 125.2, 128.6, 128.9, 129.1, 129.6, 131.3.

MS: *m*/*z* 195 [M].

2-Ethyl-3-methylindole



Ratio aniline:diol = 2:3

Yield = 49 % (PPh_3).

Yield = 57% (xantphos).

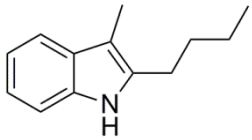
^{1}H NMR ($CDCl_3$): 1.26 (t, 3H, *J* = 7.8 Hz), 2.23 (s, 3H), 2.74 (q, 2H, *J* = 7.5 Hz), 7.04-7.14 (m, 4H), 7.22-7.28 (m, 2H), 7.46-7.54 (m, 2H), 7.68 (br s, 2H).

^{13}C NMR ($CDCl_3$): 8.3, 14.0, 19.4, 106.2, 110.1, 118.0, 119.0, 120.9, 129.4, 135.0, 136.4.

NMR data is in accordance with literature values.[260]

MS: *m*/*z*: 159 [M].

2-Butyl-3-methylindole



Ratio aniline:diol = 2:3

Yield = 54% (PPh_3).

Yield = 61% (xantphos).

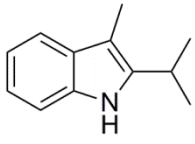
^{1}H NMR ($CDCl_3$): 0.92 (t, 3H, *J* = 7.3 Hz), 1.35 (sextet, 2H, *J* = 7.3 Hz), 1.57 (p, 2H, *J* = 7.2 Hz), 2.22 (s, 3H), 2.66 (t, 3H, *J* = 7.3 Hz), 7.09 (m, 2H), 7.20 (d, 1H, *J* = 7.1 Hz), 7.50 (d, 1H, *J* = 7.5 Hz), 7.55 (br s, 1H).

^{13}C NMR ($CDCl_3$): 8.4, 13.9, 22.4, 25.8, 31.8, 106.7, 110.1, 118.0, 118.9, 120.8, 129.3, 135.0, 135.3.

NMR data is in accordance with literature values.[261]

MS: *m*/*z* 187 [M].

2-Isopropyl-3-methylindole



Yield = 32% (PPh_3, 3 days reaction time),

Yield = 0% (xantphos, 3 days reaction time)

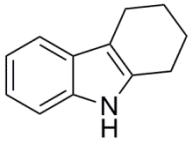
^{1}H NMR ($CDCl_3$): 1.31 (d, 6H, *J* = 7.0 Hz), 2.25 (s, 3H), 3.25 (septet, 1H, *J* = 7.0 Hz), 7.04-7.14 (m, 2H), 7.26-7.30 (m, 1H), 7.47-7.50 (m, 1H), 7.72 (br s, NH).

^{13}C NMR ($CDCl_3$): 8.4, 22.3, 25.6, 105.2, 110.2, 118.0, 119.0, 120.8, 129.4, 134.8, 140.2.

NMR data is in accordance with literature values.[262]

MS: *m*/*z* 173 [M].

1,2,3,4-Tetrahydrocarbazole



MsOH (5.0 mol%) used as additive in the reaction.

Yield = 50% (PPh_3).

Yield = 52 %(xantphos).

mp 111–113 ^{o}C (lit.[257] 114 ^{o}C, lit.[263] 116–118 ^{o}C).

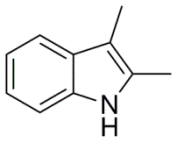
^{1}H NMR ($CDCl_3$): 1.80–1.94 (m, 4H), 2.64–2.74 (m, 4H), 7.02–7.14 (m, 2H), 7.20–7.26 (m, 1H), 7.42–7.48 (m, 1H), 7.52–7.66 (br s, 1H).

^{13}C NMR ($CDCl_3$): 21.4, 23.7, 23.8, 110.6, 110.8, 118.2, 119.5, 121.4, 128.3, 134.5, 136.1.

NMR data is in accordance with literature values.[264-266]

MS: *m*/*z* 171 [M].

2,3-dimethylindole



The yield was not determined as the compound was only used for mechanistic purposes.

^{1}H NMR ($CDCl_3$): 2.22 (s, 3H), 2.35 (s, 3H), 7.05–7.14 (m, 2H), 7.22–7.27 (m, 1H), 7.44–7.49 (m, 1H), 7.60–7.75 (br s, 1H).

^{13}C NMR ($CDCl_3$): 8.4, 11.5, 107.0, 110.0, 117.9, 118.9, 120.8, 129.3, 130.6, 135.1.

NMR data is in accordance with literature values.[265, 266]

MS: *m*/*z* 145 [M].

8 Experimental work performed at TSRI

8.1 Materials and methods

All chemicals were purchased from commercial sources. Automated synthesis was carried out on a C. S. Bio CS336X peptide synthesizer. Electrospray ionization mass spectrometry (ESI-MS) was performed on a SCIEX API-I single quadruple mass spectrometer. UV-vis was measured on a GenesysTM 6 spectrophotometer (Thermo Electron Corporation). Reverse-phase HPLC analysis and purification: HPLC buffer A is 99.9% water, 0.1% TFA and buffer B is 90% ACN, 9.9% water, 0.1% TFA. Analytical HPLCs were run on a HP1050 instrument using a Phenomenex Jupiter 4 μ Proteo 90 Å column at 0–67% B over 30 min (for peptides) or a Phenomenex Jupiter 5 μ C18 300 Å column at 20–60% B over 30 min (for ligation products). Preparative scale HPLC was run on a Waters Delta Prep 4000 instrument using a Phenomenex Jupitor 10 μ Proteo 90 Å (250 x 21.20 mm) at 25-40% or 30-55% B (for peptides) and a Phenomenex Prodigy C18 10 μ 300 Å (250 x 10.0 mm) column at 25-60% B over 60 minutes (for ligation products). Denaturation experiments were conducted on an AVIV ATF 105 automated titrating spectrofluorometer using an equilibrium time of 5 minutes for every addition, using samples of 0 M guanidine hydrochloride and 8 M guanidine hydrochloride in 0.1 M phosphate buffer at pH 6.3.

8.2 Peptide synthesis

Side-chain protection groups

Fmoc-strategy	Boc-strategy
Pbf for R	O-cyclohexyl for D and E
Trt for N and Q	Xan for Q
O*t*Bu for D and E	Clz for K
Boc for K	Bn for S and T
*t*Bu for Y	

Automated Fmoc-SPPS of H-MEYRIDRVRLFVDKLDNIAQVPRVG-OH, CI2(40-64)

PAC-PEG-PS resin (0.21 mmol Fmoc/(g resin), 476 mg, 0.1 mmol) was allowed to swell in DMF in a 100 mL round bottom flask for 30 minutes. Meanwhile, Fmoc-Gly-OH (297 mg, 1.0 mmol) was dissolved in DCM with a few drops of DMF and cooled to 0 ^{o}C. DIC (63 mg, 0.5 mmol) was added and the solution was stirred for 20 minutes after which the mixture was warmed to room temperature. The mixture was filtrated and solvents removed *in vacuo* and then redissoveld in DMF. DMAP (1.2 mg, 0.01 mmol) was added and the

mixture was added to the resin and left at room temperature with occasional swirling for 1 hour. The activated amino acid in DCM was added and the mixture was left overnight at room temperature. Decantation of solvent and addition of a new batch of activated amino acid from Fmoc-Gly-OH (297 mg, 1.0 mmol), DIC (63 mg, 0.5 mmol) and DMAP (1.2 mg, 0.01 mmol). After sitting at room temperature overnight 20 mg of resin was removed and washed with DMF, dried and transferred to a 10 mL graduated flask. 2% DBU in DMF (2 mL) was added and the mixture was gently stirred for 30 minutes. The solution was diluted to 10 mL with acetonitrile and 2 mL of this solution was taken out and diluted to 25 mL with acetonitrile. In similar way a blank sample was made without resin. Absorbance difference at 304 nm = 0.134, which correspond to (0.134 x 16.4)/18.2 = 0.12 mmol Fmoc/(g resin) remained. Therefore, the yield of the couplings were in a total of 0.12/0.21 = 60%. The rest of the resin was washed with DMF, dried and transferred to an automated Fmoc synthesizer. The remaining Fmoc-protected amino acids were coupled in a cycle consisting of 0.4 M HOBt and HCTU in DMF (2.5 mL) for coupling and 1M DIEA in DMF (1 mL) for deprotection. After coupling of the last amino acid (methionine) deprotection of Fmoc was performed manually in 20% piperidine in DMF for 20 minutes. The peptide was cleaved from the resin by treatment a TFA-cocktail (19 mL) consisting of 90% TFA, 5% DCM, 2.5% TIS and 2.5% water for 2½ hours. Solvents were removed *in vacuo* and the remaining oil was poured over ice-cold diethyl ether. After precipitation the solvent was decanted away. Mass of 3003.5 confirmed by ESI-MS. Purification by RP-HPLC.

Manual SPPS by Boc-strategy of Boc-DKPEAQIIVLPVGTIVD-thioester on resin (CI2(24-40)thioester on resin)

TAMPAL resin (800 mg, 0.4 mmol) was allowed to swell in DMF for 20 minutes and was then treated with 2.5% TIS and 2.5% water in TFA (3 x 1 minute or until clear solution is observed). Then the resin was washed with DMF three times. The amino acid (2.2 mmol) was pre-activated with 0.5 M HCTU in DMF (4 mL) and DIEA (1 mL) in DMF and shaken for 1 minute before added to the resin. After standing for 40 minutes the resin was washed twice with DMF and then treated with TFA (2 x 1 minute). The cycle was repeated starting with three times DMF wash and the next amino acids were coupled in only 20 minutes. To test if coupling was successful a Kaiser test was performed. 2-5 mg of resin was removed and added to small funnel and washed with DMF and then 1:1 DCM/MeOH. Resin was dried completely and transferred to a test tube. Two drops of a phenol/ethanol mixture, two drops of a KCN/pyridine mixture and one drop of a ninhydrin/ethanol mixture was added and diluted to 3 mL with 60% EtOH/water. The tube was heated to 100 ^{o}C for 4-5

minutes. If a blue color is observed it implies that free amine is present and therefore coupling has not been successful and must be repeated. After the final amino acid coupling the resin was washed with DMF followed by MeOH:DCM (1:1) and keept in a desiccator. Used for parallel synthesis of CI2(1-40) thioester mutants.

Manual Boc-SPPS of CI2(1-40)thioesters

Boc-DKPEAQIIVLPVGTIVD-thioester on resin (288 mg, 0.08 mmol) was allowed to swell in DMF and coupling of amino acids follow the procedure described above with 0.44 mmol amino acid, 0.5 M HCTU in DMF (0.9 mL) and DIEA (0.2 mL) in 20 minutes coupling times. However, coupling of Aib was achieved with (0.8 mmol) with HATU (1 mmol) in DMF (0.9 mL) and DIEA (15 µL) and required a coupling time of 30 minutes. After removal of the final Boc-group with TFA the resin was washed with DMF followed by MeOH:DCM. Cleaving the peptide from the resin was acheived by treatment with anhydrous liquid HF. In a typical HF cleavage reaction, 300 mg of resin was stirred in a vessel containing 1 mL of scavenger (anisole or *p*-cresol) in 10 mL of HF. The reaction was allowed to proceed at 0 ^{o}C for 1 h, and then the HF was distilled off under vacuum. The peptide-resin mixture was washed three times with ice-cold diethyl ether and filtered. The peptide was then dissolved in a buffer (27% acetonitrile, 73% water and 0.1% TFA) and lyophilized. Purification by RP-HPLC. Mass confirmed by ESI-MS.

- CI2(1-39)COSR (34 mg), M =4413.3
  LKTEWPELVGKSVAAAKKVILQDKPEAQIIVLPVGTIVD-thioester
- CI2(1-39)K18AibCOSR (38mg), M = 4370.2
  LKTEWPELVGKSVAAAK**Aib**VILQDKPEAQIIVLPVGTIVD-thioester
- CI2(1-39)K18ACOSR (41 mg), M = 4356.2
  LKTEWPELVGKSVAAAK**A**VILQDKPEAQIIVLPVGTIVD-thioester
- CI2(1-39)Q22ACOSR (30 mg), M = 4356.2
  LKTEWPELVGKSVAAAKKVIL**A**DKPEAQIIVLPVGTIVD-thioester
- CI2(1-39)Q22AibCOSR (51 mg), M =4370.2
  LKTEWPELVGKSVAAAKKVIL**Aib**DKPEAQIIVLPVGTIVD-thioester
- CI2(1-39)A15AibCOSR*
  LKTEWPELVGKSVA**Aib**AKKVILQDKPEAQIIVLPVGTIVD-thioester
- CI2(1-39)A16AibCOSR:*
  LKTEWPELVGKSVAA**Aib**KKVILQDKPEAQIIVLPVGTIVD-thioester

* made by Juan Bautista Blanco-Canosa

- CI2(1-39)Q22CycCOSR
  LKTEWPELVGKSVAAAKKVIL**Acpc**DKPEAQIIVLPVGTIVD-thioester on resin
- CI2(1-39)K18CycCOSR
  LKTEWPELVGKSVAAAK**Acpc**VILQDKPEAQIIVLPVGTIVD-thioester on resin
- CI2(1-39)A16CycCOSR
  LKTEWPELVGKSVAA**Acpc**KKVILQDKPEAQIIVLPVGTIVD-thioester on resin
- CI2(1-39)A15CycCOSR
  LKTEWPELVGKSVA**Acpc**AKKVILQDKPEAQIIVLPVGTIVD-thioester on resin
- CI2(1-39)A14CycCOSR
  LKTEWPELVGKSV**Acpc**AAKKVILQDKPEAQIIVLPVGTIVD-thioester on resin

8.3 Folding assisted ligation

CI2(1-39)COSR (8.9 mg), CI2(40-64) (8.0 mg) and thiphendol (45 µL) were dissolved in 0.1 M sodium phosphate buffer (2.205 mL) at pH 6.3. The mixture was vortexed and sonicated and placed on a rotating table overnight. Ice-cold ether was added and the vial was shaken and placed in a centrifuge. Ether decanted away and the reming oil was purified by RP-HPLC affording 4.6 mg of CI2-wt. Mass confirmed by ESI-MS.
LKTEWPELVGKSVAAAKKVILQDKPEAQIIVLPVGTIVDMEYRIDRVRLFVDKLDNIAQVPRVG

CI2(1-39)K18ACOSR (12 mg) and CI2(40-64) (11 mg) to give CI2-K18A (7.0 mg),
LKTEWPELVGKSVAAAK**A**VILQDKPEAQIIVLPVGTIVDMEYRIDRVRLFVDKLDNIAQVPRVG

CI2(1-39)K18AibCOSR (13.6 mg) and CI2(40-64) (12.2 mg) to give CI2-K18Aib (7.3 mg),
LKTEWPELVGKSVAAAK**Aib**VILQDKPEAQIIVLPVGTIVDMEYRIDRVRLFVDKLDNIAQVPRVG

CI2(1-39)Q22ACOSR (11.6 mg) and CI2(40-64) (10.1 mg) to give CI2-Q22A (7.3 mg),
LKTEWPELVGKSVAAAKKVIL**A**DKPEAQIIVLPVGTIVDMEYRIDRVRLFVDKLDNIAQVPRVG

CI2(1-39)Q22AibCOSR (18 mg) and CI2(40-64) (15 mg) to give CI2-Q22A (~10 mg),
LKTEWPELVGKSVAAAKKVIL**Aib**DKPEAQIIVLPVGTIVDMEYRIDRVRLFVDKLDNIAQVPRVG
CI2(1-39)A15AibCOSR (16 mg) and CI2(40-64) (14 mg) to give CI2-A15Aib (mass not measured),
LKTEWPELVGKSVA**Aib**AKKVILQDKPEAQIIVLPVGTIVDMEYRIDRVRLFVDKLDNIAQVPRVG

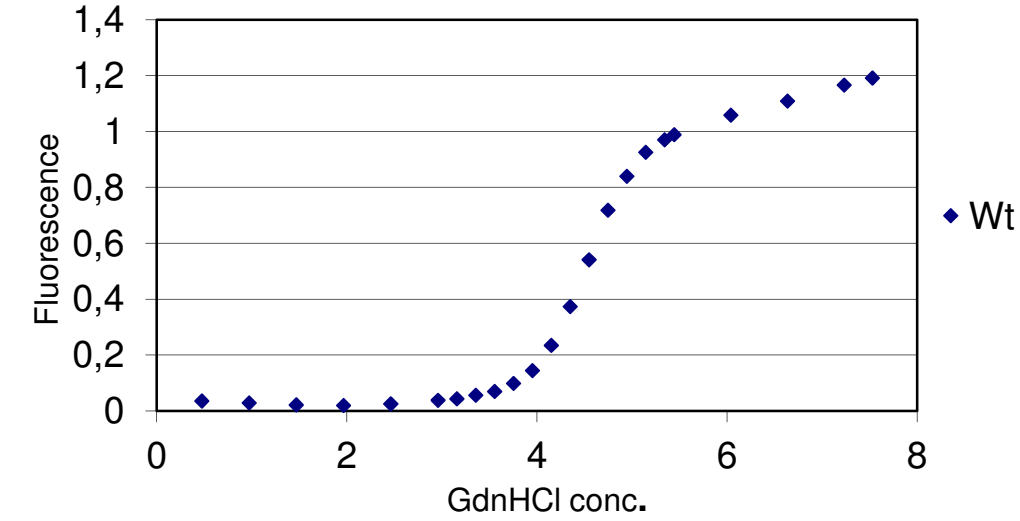
CI2(1-39)A16AibCOSR (7.1 mg) and CI2(40-64) (6.5 mg) to give CI2-A16Aib (2.8 mg),

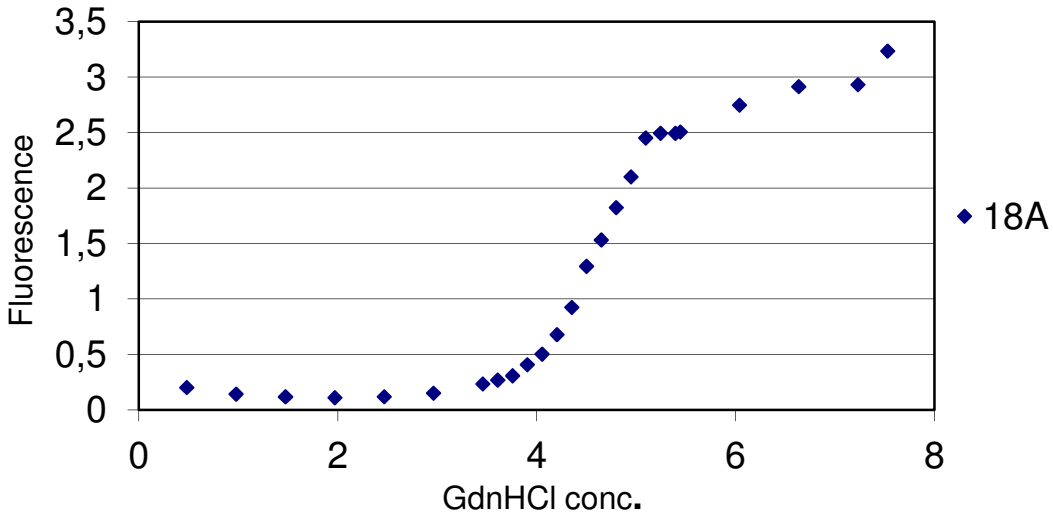
LKTEWPELVGKSVAA**Aib**KKVILQDKPEAQIIVLPVGTIVDMEYRIDRVRLFVDKLDNIAQVPRVG

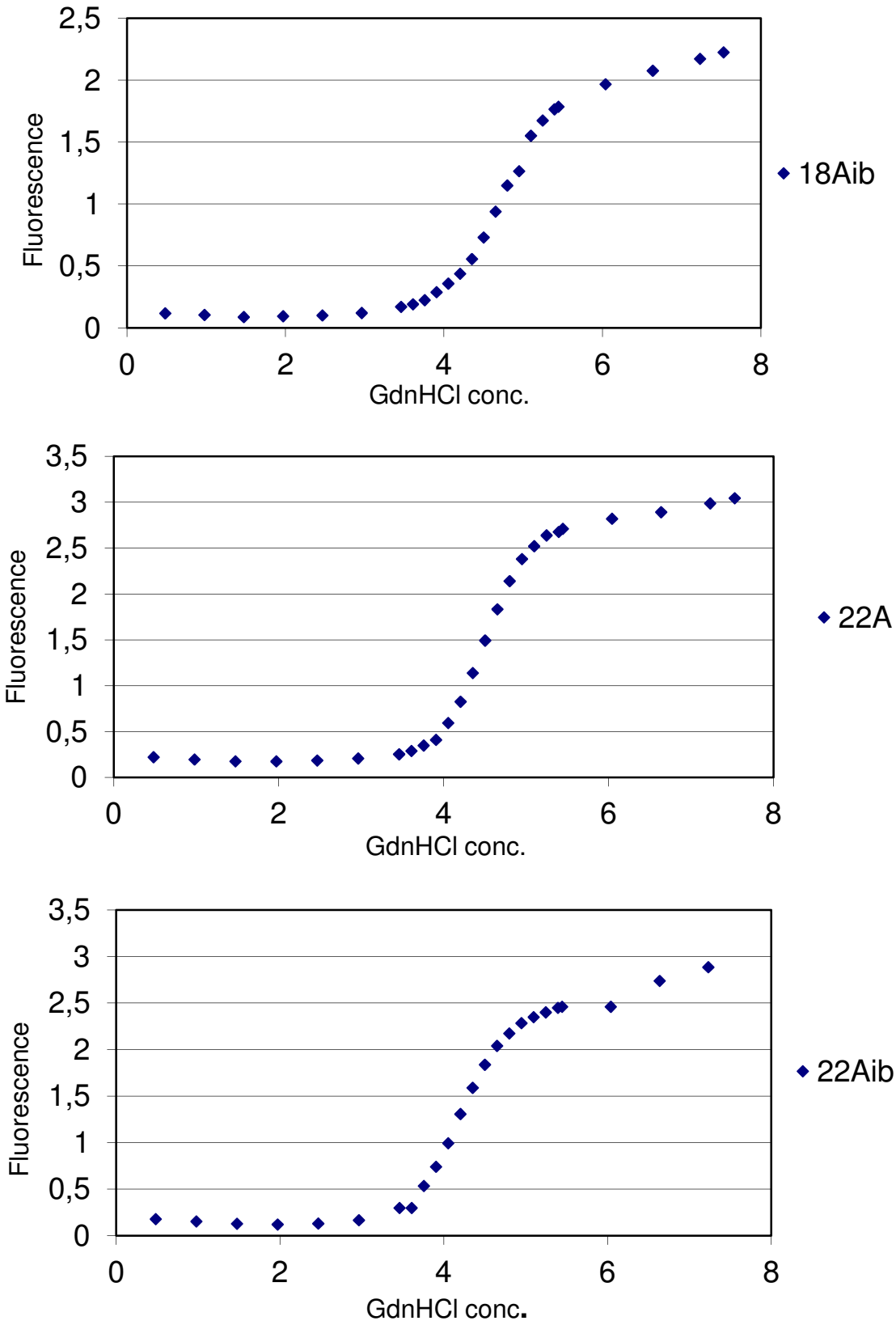
8.4 Guanidine denaturation experiments (thermodynamic and kinetic)

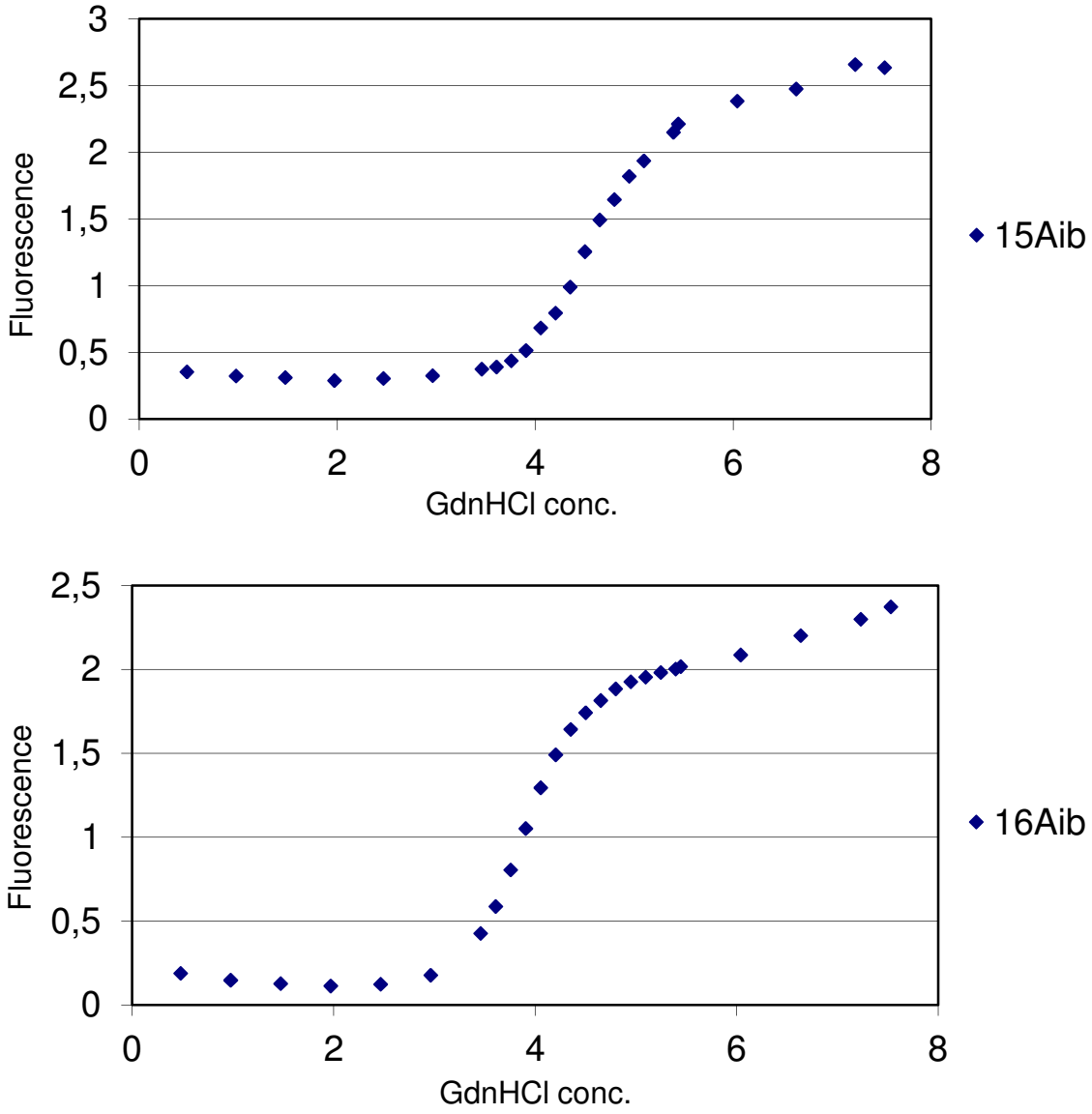
The protein (1 mg) was dissolved in 0.1 M phosphate buffer (0.5 mL) at pH 6.3. Equimolar protein was dissolved in 8 M guanidine hydrochloride in 0.1 M sodium phosphate at pH 6.3 and in 0.1 M sodium phosphate also at pH 6.3. The experiments were carried out using automated titration. Data was collected after 5 minutes equilibration time under constant stirring after each addition. Excitation wavelength was 280 nm and emission wavelength was 356 nm. All experiments were conducted at room temperature.

Thermodynamic experiments:



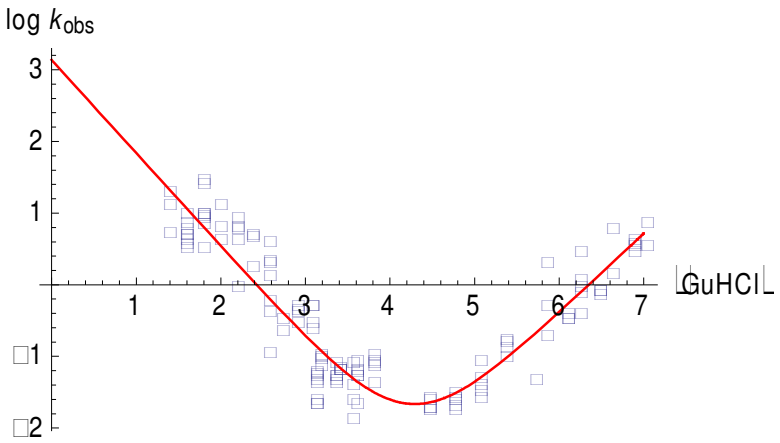



Fluorescence
2,5
2
1,5
1
0,5
0
0
2
4
6
8
GdnHCl conc.
18Aib
Fluorescence
3,5
3
2,5
2
1,5
1
0,5
0
0
2
4
6
8
GdnHCl conc.
22A
Fluorescence
3,5
3
2,5
2
1,5
1
0,5
0
0
2
4
6
8
GdnHCl conc.
22Aib

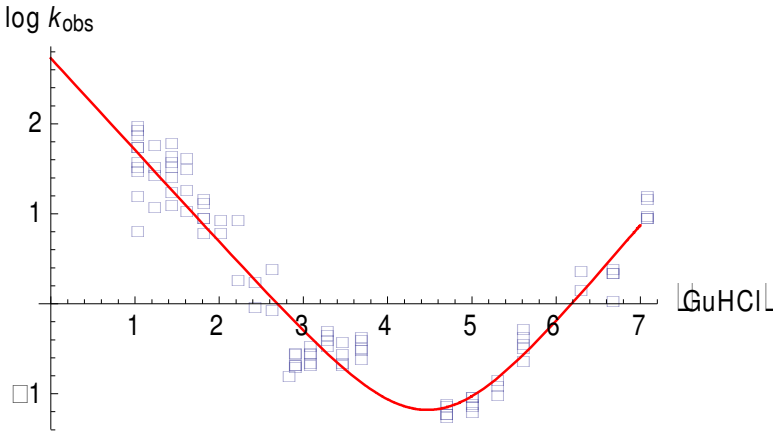


Kinetic experiments:

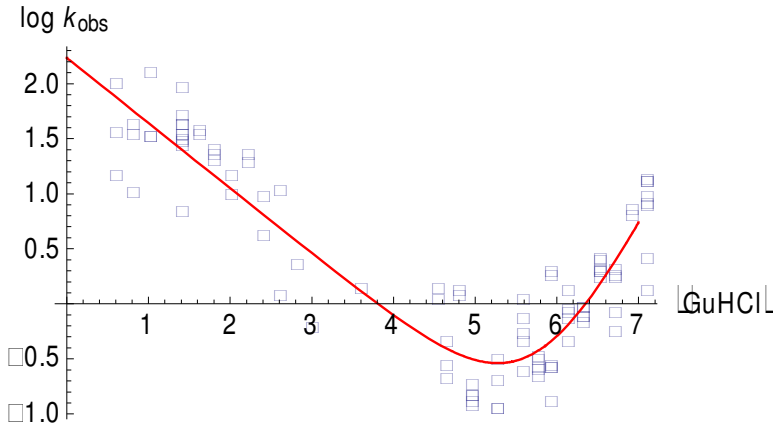
- **CI2-wt**



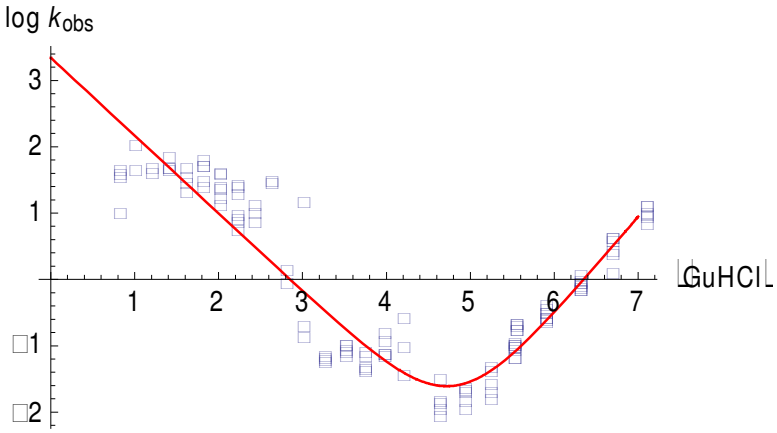
- **CI2-K18A**



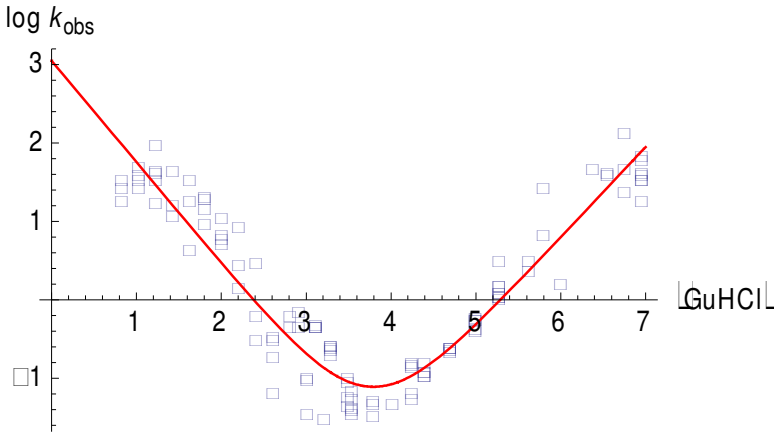
- **CI2-K18Aib**



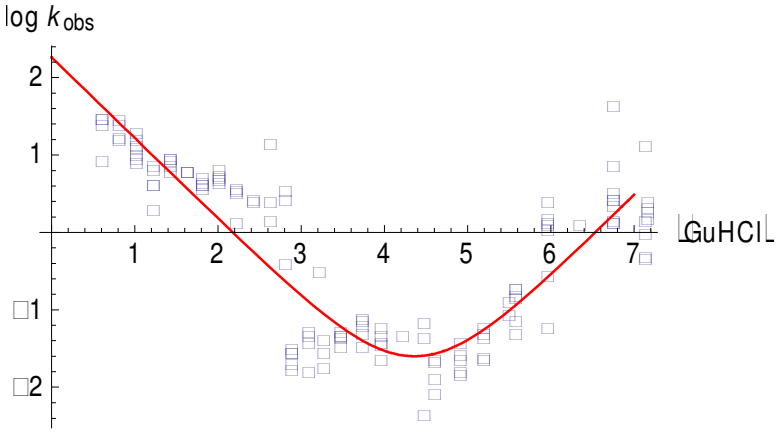
- **CI2-Q22A**



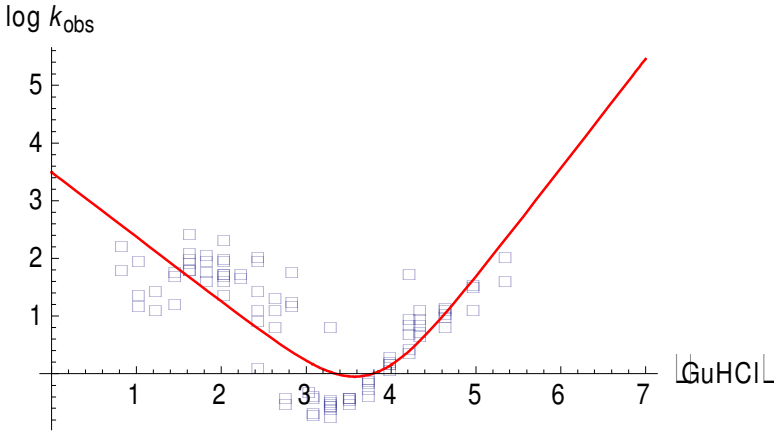
- **CI2-Q22Aib**



- **CI2-A15Aib**



- **CI2-A16Aib**



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Appendix

Publications

1) *Iridium-Catalyzed Condensation of Primary Amines To Form Secondary Amines.* Lorentz-Petersen, L. L. R.; Jensen, P.; Madsen, R. *Synthesis*, **2009**, *24*, 4110-4112

2) *Iridium- and Ruthenium-Catalyzed Synthesis of 2,3-Disubstituted Indoles from Anilines and Vicinal Diols*. Tursky, M.; Lorentz-Petersen, L. L. R.; Olsen, L. B.; Madsen, R. *Org. Biomol. Chem.* **2010**, *8*, 5576-5582

Iridium-Catalyzed Condensation of Primary Amines To Form Secondary Amines

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Abstract: Symmetric secondary amines are readily obtained by heating a neat primary amine with 0.5 mol% of bis(dichloro[η^5-pentamethylcyclopentadienyl]iridium). The products are isolated by direct distillation in good yields.

Key words: amines, dimerizations, homogeneous catalysis, hydrogen transfer, iridium

Secondary amines are an important class of molecules in chemistry and biology, and are used as intermediates in the production of pharmaceuticals, agrochemicals, dyes, and fine chemicals.[1] The most familiar methods for the synthesis of secondary amines are the N-alkylation of primary amines with alkyl halides[2] and the reduction (or alkylation) of imines.[3] The former method often suffers from problems of overalkylation, which can be solved by introducing a protecting group.[4] The latter method can be carried out by direct reductive amination, but is still a rather difficult procedure and can also lead to overalkylation. Other methods for the synthesis of secondary amines include metal-catalyzed arylation with aryl halides,[5] reductive alkylation with nitriles,[6] and reduction of secondary amides.[7]

Some procedures that are more environmentally friendly have recently been introduced, in which primary amines or ammonium salts are alkylated with alcohols in the presence of a ruthenium or an iridium catalyst.[8] The mechanism involves dehydrogenation of the alcohol to the carbonyl compound, imine formation, and reduction.

Secondary amines have also been prepared by ruthenium- or iridium-catalyzed coupling of two primary amines;[9] in this case, one of the reactants is either an aryl amine or a tertiary-alkyl amine, which allows selective coupling to occur. Self-condensation of primary amines into symmetric secondary amines in the presence of various metal catalysts has also been reported.[10] The proposed mechanism for this reaction involves dehydrogenation of the primary amine to the corresponding imine, aminal formation, elimination of ammonia, and reduction (Scheme 1). An electrocatalytic system has recently been described for the synthesis of secondary amines from primary amines; this reaction also proceeds by the mechanism shown in Scheme 1.[11]

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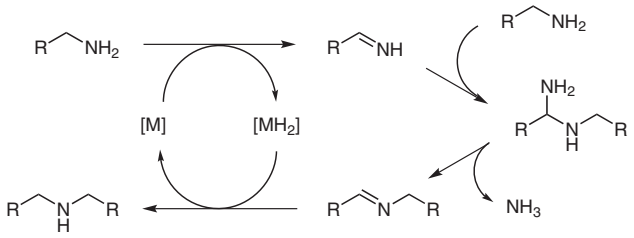
During our work on the iridium-catalyzed alkylation of amines with alcohols,[12] we sometimes observed the dimerization of the amines as a side reaction, particularly under neat conditions and with less-reactive alcohols. Here we describe a convenient method for the synthesis of secondary amines from primary amines in the presence of bis(dichloro[η^5-pentamethylcyclopentadienyl]iridium) ([Cp*$IrCl_2$]$_2$) as a catalyst.

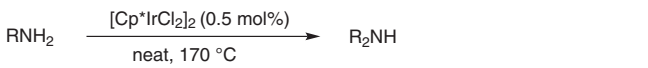


Scheme 1 Mechanism for the dimerization of amines

Our initial experiments were performed with benzylamine and 0.5 mol% of [Cp*$IrCl_2$]$_2$ in the absence of a solvent. When this mixture was heated overnight at 140 °C, significant amounts of dibenzylamine were formed, but the dimerization did not go to completion. The addition of acidic or basic additives did not accelerate the reaction. However, increasing the temperature to 170 °C resulted in a clean conversion into dibenzylamine after 18 hours. The secondary amine was isolated in 70% yield by direct distillation of the reaction mixture (Table 1, entry 1). This procedure appeared to be very convenient for the synthesis of symmetric secondary amines, so a number of other substrates were also investigated. Benzylamines containing methyl, methoxy, fluoro, chloro, or bromo substituents at the para-position underwent self-coupling in a similar manner to benzylamine, giving the corresponding dibenzylamines in 75–83% yield (entries 2–6). Note that the bromo substituent is stable under these reaction conditions, as this can serve as a handle for further functionalization reactions. 1-(2-Pyridyl)methanamine was converted into the corresponding secondary amine in 79% yield, and the reaction was not hampered by the additional heterocyclic amine group (entry 7). On the other hand, more-reactive aromatic amines, such as furfurylamine or tryptamine, decomposed under the reaction conditions. Aliphatic amines reacted significantly more slowly than did benzylic amines, presumably because of the slower initial dehydrogenation to the imine. After 18 hours, only 54% of hexylamine was converted into dihexylamine;

however, after about 70 hours, hexyl-, octyl-, and cyclohexylamine were each converted into the corresponding secondary amine in 72–73% isolated yield (entries 8–10). An attempt to perform a selective coupling between benzylamine and hexylamine was only moderately successful. Heating an equimolar mixture of the two amines with the iridium catalyst gave no more than a 40% isolated yield of *N*-benzylhexan-1-amine after flash chromatography; the remainder of the product mixture consisted of equal amounts of the two symmetric secondary amines. In all the cases shown in Table 1, only trace amounts of the corresponding tertiary amines were observed.

Table 1 Synthesis of Secondary Amines from Primary Amines



Entry	R	Time (h)	Yield (%)[a]
1	Bn	18	70
2	4-$MeC_6H_4CH_2$	18	75
3	4-$MeOC_6H_4CH_2$	18	77
4	4-$FC_6H_4CH_2$	18	83
5	4-$ClC_6H_4CH_2$	18	80
6	4-$BrC_6H_4CH_2$	18	76
7	(2-pyridyl)methyl	18	79
8	$(CH_2)_5Me$	72	72
9	$(CH_2)_7Me$	72	72
10	Cy	68	73

[a] Yield of isolated product after distillation.

In summary, we have developed a practical procedure for preparation of symmetric secondary amines from a variety of primary amines. The neat reaction conditions allow the straightforward isolation of the product by direct distillation. We believe that the method is a very attractive procedure for the synthesis of dialkylamines because of its simplicity and convenience.

^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian Mercury 300 spectrometer. The chemical shifts are reported in ppm relative to the residual deuterated solvent. Mass spectra were obtained on a Shimadzu QP5000 GC-MS instrument equipped with an Equity-1 capillary column (30 m × 0.25 mm, 0.25 μm film).

Secondary Amines; General Procedure

A primary amine (16 mmol) and $[Cp^*IrCl_2]_2$ (64 mg, 0.08 mmol) were added to a 5-mL screw-top vial that was sealed and heated to 170 °C for the time shown in Table 1, then cooled to r.t. CH_2Cl_2 (2 mL) was added, and the resulting soln was transferred to a round-bottomed flask. The CH_2Cl_2 was removed on a rotary evaporator, and the desired product was isolated by distillation in vacuo.

Dibenzylamine[13]

Bp 143–145 °C/10 mbar (Lit.[14] 113–114 °C/0.13 mbar).

^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.25–7.35 (m, 10 H), 3.78 (s, 4 H), 1.56 (s, 1 H).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 140.3, 128.3, 128.1, 126.8, 53.1.

MS (EI, 70 eV): *m/z* = 197 [M].

Bis(4-methylbenzyl)amine[15]

Bp 157–160 °C/3 mbar (Lit.[14] 135–137 °C/0.13 mbar).

^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.31–7.20 (m, 8 H), 3.83 (s, 4 H), 2.41 (s, 6 H).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 137.2, 136.3, 129.0, 128.0, 52.7, 21.0.

MS (EI, 70 eV): *m/z* = 225 [M].

Bis(4-methoxybenzyl)amine[16]

Bp 183–185 °C/3 mbar (Lit.[14] 161–163 °C/0.13 mbar).

^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.32–6.91 (m, 8 H), 3.84 (s, 6 H), 3.78 (s, 4 H).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 158.5, 132.4, 129.2, 113.6, 55.1, 52.3.

MS (EI, 70 eV): *m/z* = 257 [M].

Bis(4-fluorobenzyl)amine[15]

Bp 130–132 °C/3 mbar.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.30–6.97 (m, 8 H), 3.74 (s, 4 H).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 161.9 (d, *J* = 245 Hz), 135.9 (d, *J* = 3.2 Hz), 129.6 (d, *J* = 7.8 Hz), 115.1 (d, *J* = 21 Hz), 52.3.

MS (EI, 70 eV): *m/z* = 233 [M].

Bis(4-chlorobenzyl)amine[13]

Bp 177–181 °C/3 mbar (Lit.[17] 230 °C/20 mbar).

^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.30–7.26 (m, 8 H), 3.73 (s, 4 H), 1.56 (s, 1 H).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 138.5, 132.6, 129.4, 128.4, 52.2.

MS (EI, 70 eV): *m/z* = 266 [M].

Bis(4-bromobenzyl)amine[16]

Mp 46–48 °C (Lit.[16] 48–49 °C); bp 145–147 °C/0.04 mbar.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.44 (d, *J* = 8.2 Hz, 4 H), 7.20 (d, *J* = 8.2 Hz, 4 H), 3.72 (s, 4 H).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 139.1, 131.4, 129.8, 120.7, 52.3.

MS (EI, 70 eV): *m/z* = 355 [M].

Bis[(2-pyridyl)methyl]amine[18]

Bp 107–110 °C/0.09 mbar (Lit.[18] 130–135 °C/0.13 mbar).

^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.50–8.48 (m, 2 H), 7.56 (td, *J* = 1.7 and 7.7 Hz, 2 H), 7.28 (d, *J* = 7.8 Hz, 2 H), 7.10–7.06 (m, 2 H), 3.91 (s, 4 H), 2.52 (s, 1 H).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 159.5, 149.1, 136.3, 122.1, 121.8, 54.6.

MS (EI, 70 eV): *m/z* = 200 [M + H].

Dihexylamine[19]

Bp 85–86 °C/3 mbar (Lit.[20] 75 °C/1.3 mbar).

^{1}H NMR (300 MHz, $CDCl_3$): δ = 2.59 (t, *J* = 7.6 Hz, 4 H), 1.48 (m, 4 H), 1.29 (m, 12 H), 0.89 (t, *J* = 6.5 Hz, 6 H).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 50.1, 31.7, 30.1, 27.0, 22.5, 13.9.

MS (EI, 70 eV): *m/z* = 185 [M].

Dioctylamine[19]
Bp 139–141 °C/3 mbar (Lit.[21] 145–155 °C/4 mbar).

^{1}H NMR (300 MHz, $CDCl_3$): δ = 2.50 (t, *J* = 7.3 Hz, 4 H), 1.39 (m, 4 H), 1.20 (br s, 20 H), 0.79 (t, *J* = 6.7 Hz, 6 H).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 50.1, 31.7, 30.1, 29.4, 29.2, 27.3, 22.5, 13.9.

MS (EI, 70 eV): *m/z* = 241 [M].

Dicyclohexylamine[8d]
Bp 93–95 °C/5 mbar (Lit.[10e] 79–81 °C/0.5 mbar).

^{1}H NMR (300 MHz, $CDCl_3$): δ = 2.55 (m, 2 H), 1.91–1.81 (m, 4 H), 1.76–1.67 (m, 4 H), 1.65–1.56 (m, 2 H), 1.32–0.95 (m, 10 H).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 52.9, 34.2, 26.1, 25.2.

MS (EI, 70 eV): *m/z* = 181 [M].

Acknowledgment

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Iridium- and ruthenium-catalysed synthesis of 2,3-disubstituted indoles from anilines and vicinal diols†

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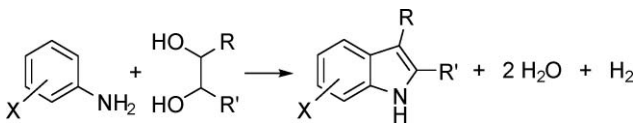
A straightforward and atom-economical method is described for the synthesis of 2,3-disubstituted indoles. Anilines and 1,2-diols are condensed under neat conditions with catalytic amounts of either $[Cp^*IrCl_2]_2$/MsOH or $RuCl_3 \cdot xH_2O$/phosphine (phosphine = PPh_3 or xantphos). The reaction does not require any stoichiometric additives and only produces water and dihydrogen as byproducts. Anilines containing methyl, methoxy, chloro and fluoro substituents can participate in the cyclocondensation. Meta-substituted anilines give good regioselectivity for 6-substituted indoles, while unsymmetrical diols afford excellent regioselectivity for the indole isomer with an aryl or large alkyl group in the 2-position. The mechanism for the cyclocondensation presumably involves initial formation of the α-hydroxyketone from the diol. The ketone subsequently reacts with aniline to generate the α-hydroxyimine which rearranges to the corresponding α-aminoketone. Acid- or metal-catalysed electrophilic ring-closure with the release of water then furnishes the indole product.

Introduction

The indole skeleton is one of the most important heterocyclic ring systems which is found in many natural products[1] and biologically active molecules.[2] Substituted indoles are capable of binding to a number of receptors with high affinity and the indole substructure is found in a variety of different drugs.[3] This has stimulated intense research into the chemical synthesis of indoles for more than a century.[4] The Fischer indole synthesis from 1883[5] is still a widely applied method where aryl hydrazines are reacted with enolisable aldehydes/ketones to afford the heterocycle after a sigmatropic rearrangement of the corresponding hydrazone.[6] Another classical but less commonly used procedure is the Bischler indole synthesis from 1892[7] where anilines are alkylated with α-haloketones and the resulting α-anilinoketones then cyclised to the target molecule.[8] More recently, a variety of new procedures have been developed for assembling the indole ring system particularly by the use of various palladium-catalysed cyclisations.[9] However, the starting materials are often 1,2-disubstituted aromatic compounds such as 2-haloanilines, which may not be widely available, but have to be prepared in separate steps. A more straightforward protocol with simple starting materials involve condensation of anilines with 1,2-diols to afford indoles after liberating two molecules of water and one molecule of dihydrogen (Scheme 1). This procedure is also a very environmentally friendly and atom-economical method for synthesis of the heterocycle.[10] The reaction has previously been achieved with anilines and $RuCl_2(PPh_3)_3$ in dioxane at 180 °C[11] or with 1-naphthylamine and $IrCl_3 \cdot 3H_2O$/BINAP in mesitylene at 169 °C under air.[12] However, in both cases a significant excess of the arylamine is employed. A related indole synthesis has been described with



Scheme 1 Indole synthesis from anilines and 1,2-diols.

anilines, alkanolammonium chlorides and $RuH_2(PPh_3)_4$, but in this case an additional stoichiometric amount of $SnCl_2 \cdot 2H_2O$ is required.[13]

Recently, we have shown that piperazines can be formed by cyclocondensation of primary amines with 1,2-diols in the presence of the iridium catalyst $[Cp^*IrCl_2]_2$.[14] The C–N bond is generated by dehydrogenation of the alcohol to the carbonyl compound followed by imine formation and hydrogenation to the product amine with the liberated dihydrogen from the first step.[15,16] We have also shown that oxindoles can be alkylated in the 3-position with alcohols in the presence of $RuCl_3 \cdot xH_2O$ and PPh_3.[17] We speculated that with anilines the iridium or the ruthenium catalyst would mediate both the C–N and the C–C bond formation to furnish the indole skeleton. Herein, we describe an expedient procedure for synthesis of substituted indoles by cyclocondensation of anilines with 1,2-diols in the presence of either $[Cp^*IrCl_2]_2$ or $RuCl_3 \cdot xH_2O$/phosphine.

Results and discussion

The initial experiments were carried out with aniline, butane-2,3-diol and 1% $[Cp^*IrCl_2]_2$ in a toluene solution. In the absence of any other additives very little conversion was observed at 110 °C while increasing the temperature to 170 °C led to a very complex mixture. With 5% K_2CO_3, which is known to co-catalyse the C–N bond formation,[15] a complex mixture of products was still obtained with no visible cyclisation to the indole. Our previous work had shown that the C–N bond formation could also be mediated by an acidic co-catalyst.[14] We reasoned that the acid would also

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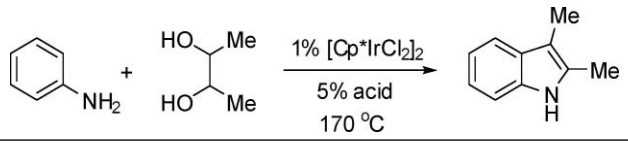
† Electronic supplementary information (ESI) available: NMR spectra for all products. See DOI: 10.1039/c0ob00106f

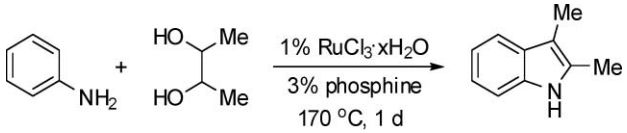
Table 1 Synthesis of 2,3-dimethylindole with different acid co-catalysts[a]



Entry	Acid	Reaction time	Yield[b]
1	H_2SO_4[c]	1 d	48%
2	H_2SO_4[c]	4 d	59%
3	none	2 d	16%
4	conc. HCl	5 d	53%
5	conc. HBr	5 d	53%
6	ZnI_2	1 d	18%
7	$MgBr_2$	2 d	53%
8	$AlCl_3$	3 d	58%
9	TMSOTf	2 d	52%
10	$BF_3{\cdot}OEt_2$	2 d	70%
11	MsOH	2 d	76%
12	conc. H_3PO_4	3 d	75%
13	AcOH	2 d	20%
14	CF_3CO_2H	2 d	46%
15	CF_3SO_3H	2 d	59%

[a] Performed with aniline (90 µL, 1 mmol), butane-2,3-diol (90 µL, 1 mmol), $[Cp^*IrCl_2]_2$ (8 mg, 0.01 mmol) and acid (0.05 mmol) in a closed vial at 170 °C. [b] Isolated yield. [c] With 2.5% H_2SO_4.

Table 2 Synthesis of 2,3-dimethylindole with ruthenium trichloride and different phosphines[a]



Entry	Phosphine	Yield[b]
1	PPh_3	65% (60%)
2	PCy_3	27% (20%)
3	$P(OEt)_3$	11%
4	$P(2\text{-furyl})_3$	9%
5	$(4\text{-}MeOC_6H_4)_3P$	64%
6	$(4\text{-}FC_6H_4)_3P$	70%
7	DPEphos[c]	60%
8	BINAP[c]	35%
9	dppe[c]	38%
10	dppp[c]	70%
11	dppb[c]	73% (66%)
12	dpppentane[c]	62%
13	dppf[c]	53%
14	xantphos[c]	76% (71%)

[a] Performed with aniline (90 µL, 1 mmol), butane-2,3-diol (90 µL, 1 mmol), $RuCl_3{\cdot}xH_2O$ (2.3 mg, 0.01 mmol) and phosphine (0.03 mmol) in a closed vial at 170 °C. [b] GC yields (isolated yields in parenthesis). [c] 1.5% bidentate phosphine.

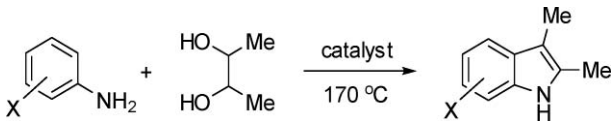
facilitate the C–C bond formation to the indole. Indeed, when the substrates were reacted in toluene at 170 °C with 5% sulfuric acid, 2,3-dimethylindole was obtained as the main product. The reaction was rather slow and only gave 34% yield after 2 days. The main problem seems to be precipitation of the ammonium salt between aniline and the acid. Under neat conditions, however, a homogeneous mixture was obtained and a faster conversion was observed giving rise to the indole in 48–59% isolated yield (Table 1, entries 1 and 2). The reaction was performed in a closed vial with equimolar amounts of aniline and butane-2,3-diol and catalytic amounts of the additives. Although, dihydrogen is released and the pressure increases, no reduction to 2,3-dimethylindoline was observed. Not surprisingly, the reaction did not proceed in the absence of the iridium catalyst.

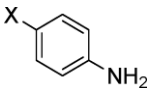
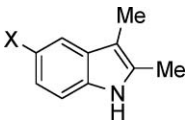
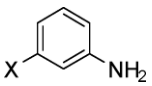
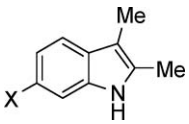
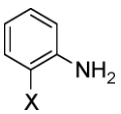
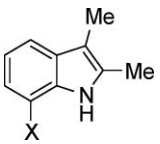
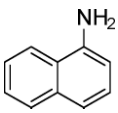
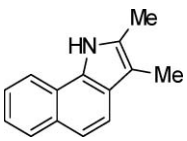
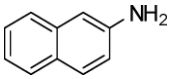
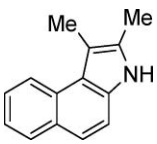
Even though sulfuric acid is a very convenient co-catalyst, the reaction was still rather slow and gave rise to a few minor byproducts. Therefore, a number of experiments were performed with different acids in order to identify the optimum co-catalyst. The acid was necessary since the reaction otherwise gave a complex mixture from which the indole could only be isolated in a low yield (entry 3). Concentrated hydrochloric acid and hydrobromic acid led to essentially the same result as sulfuric acid while several Lewis acids gave faster conversion, but without significantly improving the yield (entries 4–10). The best result was obtained with methanesulfonic acid which gave very clean conversion into the indole with no visible byproducts or starting material remaining according to GC (entry 11). Concentrated phosphoric acid also afforded a good yield, but the reaction was slower while other Brønsted acids were less effective (entries 12–15). As a result the favoured catalyst system consisted of 1% $[Cp^*IrCl_2]_2$ and 5% methanesulfonic acid.

Since ruthenium is significantly cheaper than iridium it was decided also to investigate the cyclocondensation in the presence of various ruthenium catalysts. Based on our previous experience with alkylation of oxindole[17] we focused our attention on ruthenium trichloride and various phosphines in the absence of a solvent. The first experiment was performed with aniline, butane-2,3-diol, $RuCl_3{\cdot}xH_2O$ (1%), PPh_3 (3%) and methanesulfonic acid (5%). After heating the mixture to 170 °C for 1 day, 2,3-dimethylindole was isolated in ~50% yield. When the experiment was repeated in the absence of the sulfonic acid the indole was obtained in 60% yield (Table 2, entry 1). Contrary to the iridium experiment the acid is not necessary in this case to promote the cyclisation. A number of phosphines were then investigated to identify the optimum ligand for the reaction (entries 2–14).

The best results were achieved with the two bidentate ligands dppb and xantphos which gave 66 and 71% isolated yield, respectively. The ratio between ruthenium and phosphorus was further investigated with PPh_3 and dppb, but in both cases the 1 : 3 ratio was found to give the highest yield. However, when repeating the same experiment several times we did in some cases observe a variation in the yield. This appears to be caused by differences in the size of the ruthenium trichloride crystals. The active catalyst is presumably a ruthenium(II) complex generated *in situ* by reduction of ruthenium trichloride with the phosphine. Thus it seems that this process may vary slightly from one experiment to another depending on the ruthenium trichloride batch. When the cyclocondensation was performed with the more soluble $RuCl_2(PPh_3)_3$ complex, consistent yields around 50% were obtained. Due to the convenience of the *in situ* generated catalyst we opted for a solution where this could be generated in a more reproducible way. It turned out that if the reaction mixture was stirred at 110 °C for 1 h and then heated to 170 °C more consistent results were obtained. At 110 °C the cyclocondensation does not proceed, but ruthenium trichloride reacts with the phosphine and the active catalyst is generated. Subsequent heating to the reaction

Table 3 Synthesis of indoles from substituted anilines and butane-2,3-diol[a]



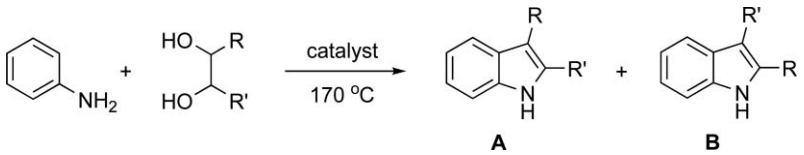
Entry	Aniline	X	Indole	Yield[b] $[Cp^*IrCl_2]_2$	Yield[b] $RuCl_3/PPh_3$	Yield[b] $RuCl_3$/xantphos
1		Me		61%	48%	50%
2		OMe		59%	56%	53%
3		Cl		66%	57%	69%
4		F		46%	45%	52%
5		Me		34%	49%	51%
6		OMe		64%	48%	58%
7		Cl		47%[c]	57%[d]	69%[d]
8		F		47%[e]	48%	52%
9		Me		65%	72%	87%
10		OMe		41%	41%	34%
11		—		65%	60%	72%[f]
12		—		76%	58%	63%[f]

[a] See experimental section for reaction procedures. [b] Isolated yield. [c] Isolated as a 4 : 1 mixture of the 6-chloro and the 4-chloro isomer. [d] Isolated as a 6 : 1 mixture of the 6-chloro and the 4-chloro isomer. [e] Minor amounts of the corresponding 4-fluoroindole was observed, but not isolated. [f] Reaction time 2 days.

temperature furnishes a more reproducible yield of the indole product .

With these optimised conditions in place the stage was now set to explore the substrate scope and limitation of the cyclocondensation method. For each substrate the reaction was performed with both the iridium catalyst ($[Cp^*IrCl_2]_2$/MsOH) and with the ruthenium catalyst ($RuCl_3/PPh_3$ and $RuCl_3$/xantphos). First, regioselectivity and functional groups in the aniline were investigated by reacting various substituted anilines with butane-2,3-diol (Table 3). Methyl and methoxy substituents were compatible with the reaction conditions and yielded the corresponding indoles without any major byproducts (entries 1, 2, 5, 6, 9 and 10). Chloro and fluoro substituents were allowed in the *para* and the *meta* positions (entries 3, 4, 7 and 8) while *o*-chloro- and *o*-fluoroaniline reacted sluggishly and gave less than 25% yield of the corresponding indole (results not shown). With the chloroanilines small amounts of 2,3-dimethylindole was observed as a byproduct, but not isolated. Bromo and boronic ester substituents, on the other hand, were completely reduced off with both the iridium and the ruthenium catalyst and carboxylic acids underwent decarboxylation. Methyl ester groups were partially cleaved off while anilines with cyano, dimethylamino, acetamido, nitro or trifluoromethyl substituents either decomposed or reacted very poorly. Meta-substituted anilines gave a good regioselectivity in the cyclisation to the *para* position (entries 5–8). The same regioselectivity is observed for the sigmatropic rearrangement in the Fischer indole synthesis when the directing group is *ortho-para* directing.[6] The two naphthyl amines gave good yields of the corresponding benzindoles (entries 11 and 12) while 2-aminopyridine gave a complex mixture and 4-aminopyridine did not react. In all cases, the three different catalysts gave comparable yields of the indole product. However, the reactions with the ruthenium catalysts were performed in a shorter time and with a lower catalyst loading than with the iridium catalyst and the ruthenium system is therefore recommended for general use. With this system the xantphos ligand

Table 4 Synthesis of indoles from aniline and various diols[a]



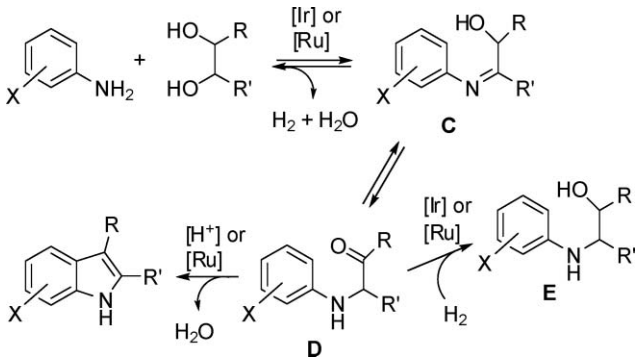
Entry	R	R′	Yield[b] $[Cp^*IrCl_2]_2$	Yield[b] $RuCl_3/PPh_3$	Yield[b] $RuCl_3$/xantphos
1	Me	Et	70% (5 : 1)[c]	49% (1 : 0)[c,d]	57% (1 : 0)[c,d]
2	Me	*n*Bu	65% (7 : 1)[c]	54% (7 : 1)[c]	61% (1 : 0)[c,d]
3	Me	*i*Pr	58% (1 : 0)	32% (1 : 0)[e]	—
4	Me	Ph	31% (1 : 0)[c]	27% (1 : 0)	28% (1 : 0)
5	-$(CH_2)_4$-		53%	50%[f]	52%[f]
6	Ph	Ph	29%	—	—

[a] See experimental section for reaction procedures. [b] Isolated yield (**A** : **B** ratio in parenthesis). [c] Aniline : diol ratio 2 : 3. [d] Minor isomer not isolated. [e] Reaction time 3 days. [f] 3% MsOH was also added.

usually gives a slightly better yield than the triphenylphosphine ligand.

The regioselectivity was also investigated with respect to the diol by reacting different diols with aniline in the presence of the iridium and the ruthenium catalyst. Remarkably, unsymmetric diols gave excellent selectivity for the indole isomer where the large substituent is placed in the 2-position (Table 4, entries 1–4). With the iridium catalyst pentane-2,3-diol and heptane-2,3-diol gave the two indoles in ratios of 5 : 1 and 7 : 1, respectively (entry 1 and 2). More unsymmetrical diols afforded exclusively the isomer with the aryl or large alkyl group in the 2-position (entries 3 and 4). A cyclic diol and a diol with two aryl groups also reacted with aniline although the latter gave a lower yield due to the instability of the diol under the reaction conditions (entries 5 and 6). With the ruthenium catalyst cyclohexane-1,2-diol reacted quite sluggishly with aniline and only afforded the tetrahydrocarbazole in low yield. However, by co-catalysing the reaction with methanesulfonic acid the yield increased to the same level as with the iridium catalyst (entry 5). Ethylene glycol and diols containing a primary alcohol gave complex mixtures with both the iridium and the ruthenium catalyst. The reason may be the poor stability of the products under the reaction conditions. Control experiments with the iridium catalyst showed that indole, 2-methylindole and 3-methylindole all underwent further reactions when exposed to the diol and methanesulfonic acid.

The mechanism for the condensation presumably involves initial formation of the α-hydroxyketone which then reacts with the aniline to furnish the imine **C** (Scheme 2). The α-hydroxyimine can either isomerise to the corresponding α-aminoketone **D** or react with the catalyst and hydrogen to generate the α-aminoalcohol **E**. Since α-hydroxyimines are known to isomerise readily in refluxing benzene[18] the former reaction is the most likely pathway. This was further confirmed by preparing α-aminoalcohol **E** (with R,R′ = -$(CH_2)_4$-) from aniline and cyclohexene oxide. When this compound was treated with $[Cp^*IrCl_2]_2$ and methanesulfonic acid, a complex mixture was observed with only little indole formation indicating that the α-aminoalcohol **E** is not part of the main reaction pathway. The same experiment was carried out with α-aminoalcohol **E** (with R = R′ = Me) and $RuCl_3 \cdot xH_2O/PPh_3$. In this case, the reaction did not go to completion in 24 h and afforded an equal mixture of aniline and 2,3-dimethylindole, which again



Scheme 2 Suggested mechanism for indole formation.

indicates that **E** is not part of the main pathway. When the reactions in Table 1–3 were monitored by GC-MS, the α-aminoalcohol **E** was observed, but mainly in cases where a weak acid or an electron-withdrawing group on the aniline made the cyclisation difficult. This suggests that α-aminoalcohol **E** is formed in a side reaction by reduction of α-aminoketone **D** and the success of the overall reaction depends on the ability of **D** to undergo electrophilic ring-closure under the acidic conditions. α-Aminoketone **D** (with R = R′ = Me) can be prepared by reacting aniline with acetoin in the absence of an acid.[19] When this aminoketone was heated with 5% methanesulfonic acid at 170 °C, the conversion into the indole was complete in 15 min while the reaction at 100 °C took about 2 h. When α-aminoketone **D** (with R = R′ = Me) was treated with 1% $RuCl_3 \cdot xH_2O/PPh_3$ at 170 °C complete conversion into the indole was observed in less than 2 h. It is unlikely, that the reaction goes through an indoline followed by dehydrogenation to the indole. Control experiments have shown that the iridium catalyst is rather slow at dehydrogenating indoline to indole (2 days at 170 °C) and since indolines are not observed by GC-MS during the course of the reaction they are most likely not intermediates in the cyclocondensation.

The excellent regioselectivity with the unsymmetrical diols in Table 4 is most likely determined in the last cyclisation step. Aminoketone **D** is also formed as an intermediate in the Bischler indole synthesis[7,8] and related transformations[20] and in these cases the cyclisation to the indole is not regiospecific, but occurs in such a way that the aryl or large alkyl group is placed in the 2-position

of the indole. For example, 2-phenylindole is formed exclusively when aniline is reacted with phenacyl bromide[21] indicating a complete rearrangement of the initially formed aminoketone. Therefore, aminoketone **D** will only be prone to cyclisation into the indole when R is a small alkyl group, and if this is not the case isomerisation into the opposite regioisomer and subsequent cyclisation will be more favourable.

Conclusion

In summary, we have developed a simple and atom-economical synthesis of 2,3-disubstituted indoles by cyclocondensation of equimolar amounts of anilines and 1,2-diols in the presence of catalytic amounts of $[Cp^*IrCl_2]_2$/MsOH or $RuCl_3$/phosphine. The reaction does not require any solvent or stoichiometric additives and only produces water and dihydrogen as byproducts.

Experimental

GC yields were obtained on a Shimadzu GC2010 instrument equipped with an Equity™ 1 column (15 m × 0.1 mm, 0.1 μm film) using naphthalene as the internal standard. Melting points are uncorrected. Solvents used for chromatography were of HPLC grade. Thin layer chromatography was performed on aluminium plates coated with silica gel 60. Visualisation was done by UV or by dipping in a solution of cerium(IV)sulfate (2.5 g) and ammonium molybdate (6.25 g) in 10% sulfuric acid (250 mL) followed by charring with a heatgun. Flash chromatography was performed with silica gel 60 (35–70 μm). NMR spectra were recorded on a Varian Mercury 300 instrument. Chemical shifts were measured relative to the signals of residual $CHCl_3$ (7.26 ppm)/$CDCl_3$ (77.0 ppm) or DMSO-d_5 (2.50 ppm)/DMSO-d_6 (39.4 ppm). Mass spectrometry was performed by direct inlet on a Shimadzu GCMS-QP5000 instrument. High resolution mass spectra were recorded at the Department of Physics and Chemistry, University of Southern Denmark.

General procedure for iridium-catalysed preparation of indoles. In an oven-dried heavy-walled vial (11 mL) equipped with a screw cap were placed the aniline (1 mmol), the diol (1 mmol), $[Cp^*IrCl_2]_2$ (8 mg, 0.01 mmol) and MsOH (3 μL, 0.05 mmol) under an argon atmosphere. The vial was closed and immediately placed in an aluminium block pre-heated to 170 °C and heated for 2 days. The mixture was quenched with triethylamine (15 μL, 0.1 mmol) and purified by column chromatography on silica gel (hexane–CH_2Cl_2 2 : 1 or heptane/EtOAc 5 : 1) to afford the desired indole.

General procedure for ruthenium-catalysed preparation of indoles. In an oven-dried heavy-walled vial equipped with a screw cap were placed the aniline (2 mmol), the diol (2 mmol), $RuCl_3 \cdot xH_2O$ (4.8 mg, 0.02 mmol) and triphenylphosphine (15.7 mg, 0.06 mmol) (or xantphos (17.4 mg, 0.03 mmol)). The vial was closed and placed in an aluminium block for 1 h at 110 °C and then heated to 170 °C for 24 h. The reaction mixture was worked up as described above.

2,3-Dimethylindole. mp 98–101 °C (lit.[19] 106–108 °C, lit.[22] 107–108 °C). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.22 (s, 3H), 2.35 (s, 3H), 7.05–7.14 (m, 2H), 7.22–7.27 (m, 1H), 7.44–7.49 (m, 1H), 7.60–7.75 (br s, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 8.4, 11.5, 107.0, 110.0, 117.9, 118.9, 120.8, 129.3, 130.6, 135.1. MS: m/z 145 [M]. NMR data are in accordance with literature values.[23,24]

2,3,5-Trimethylindole. mp 115–120 °C (lit.[22] 118–120 °C, lit.[19] 120–122 °C). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.19 (s, 3H), 2.33 (s, 3H), 2.44 (s, 3H), 6.92 (dd, 1H, J = 8.1, 1.5 Hz), 7.13 (d, 1H, J = 8.1 Hz), 7.24 (s, 1H), 7.55 (br s, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 8.4, 11.5, 21.5, 106.5, 109.6, 117.7, 122.3, 128.1, 129.6, 130.7, 133.4. MS: m/z 159 [M]. NMR data are in accordance with literature values.[22]

5-Methoxy-2,3-dimethylindole. mp 105–108 °C (lit.[19] 110–112 °C, lit.[25] 106–108 °C). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.19 (s, 3H), 2.34 (s, 3H), 3.86 (s, 3H), 6.76 (dd, 1H, J = 8.7, 2.4 Hz), 6.92 (d, 1H, J = 2.4 Hz), 7.13 (d, 1H, J = 8.7 Hz), 7.58 (br s, 1H). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 2.11 (s, 3H), 2.27 (s, 3H), 3.73 (s, 3H), 6.60 (dd, 1H, J = 8.6, 2.4 Hz), 6.83 (d, 1H, J = 2.3 Hz), 7.09 (d, 1H, J = 8.6 Hz), 10.45 (s, NH). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 8.5, 11.6, 55.9, 100.3, 106.9, 110.4, 110.6, 129.8, 130.2, 131.6, 153.8. ^{13}C NMR (DMSO-d_6, 75 MHz): δ 8.4, 11.3, 55.2, 99.6, 104.8, 109.4, 110.7, 129.2, 130.1, 132.0, 152.8. MS: m/z 175 [M]. ^{1}H NMR data are in accordance with literature values.[25,26]

5-Chloro-2,3-dimethylindole. mp 138–140 °C (lit.[19,24] 142–143 °C, lit.[27] 141–142 °C). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.16 (s, 3H), 2.32 (s, 3H), 7.00–7.14 (m, 2H), 7.40–7.41 (m, 1H), 7.62 (br s, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 8.3, 11.5, 106.9, 110.9, 117.5, 120.9, 125.1, 130.5, 132.3, 133.4. MS: m/z 179 [M]. NMR data are in accordance with literature values.[24]

5-Fluoro-2,3-dimethylindole. mp 98–99 °C (lit.[28] 98 °C). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.17 (s, 3H), 2.33 (s, 3H), 6.83 (td, 1H, J = 11.7, 2.4 Hz), 7.06–7.16 (m, 2H), 7.50–7.70 (br s, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 8.4, 11.6, 102.9 (d, J = 23 Hz), 107.4 (d, J = 4.5 Hz), 108.7 (d, J = 26 Hz), 110.4 (d, J = 9.6 Hz), 129.8 (d, J = 9.5 Hz), 131.5, 132.8, 157.7 (d, J = 233 Hz). MS: m/z 163 [M]. ^{1}H NMR data are in accordance with literature values.[26]

2,3,6-Trimethylindole. mp 98–104 °C (lit.[27] 117–118 °C). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.19 (s, 3H), 2.30 (s, 3H), 2.43 (s, 3H), 6.90 (d, 1H, J = 8.1 Hz), 7.00 (s, 1H), 7.35 (d, 1H, J = 8.1 Hz), 7.43 (br s, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 8.6, 11.5, 21.7, 106.8, 110.2, 117.7, 120.6, 127.3, 129.9, 130.5, 135.7. MS: m/z 159 [M].

6-Methoxy-2,3-dimethylindole. mp 129–132 °C (lit.[29] 130 °C). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.21 (s, 3H), 2.31 (s, 3H), 3.85 (s, 3H), 6.75 (d, 1H, J = 2.2 Hz), 6.78 (dd, 1H, J = 8.5, 2.2 Hz), 7.35 (d, 1H, J = 8.5 Hz), 7.48 (br s, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 8.5, 11.5, 55.7, 94.3, 106.8, 108.2, 118.4, 123.9, 129.2, 135.7, 155.7. MS: m/z 175 [M]. ^{1}H NMR data are in accordance with literature values.[29]

6-Chloro-2,3-dimethylindole. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.21 (s, 3H), 2.34 (s, 3H), 7.06 (dd, 1H, J = 8.4, 1.8 Hz), 7.19 (d, 1H, J = 1.8 Hz), 7.36 (d, 1H, J = 8.4 Hz). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 8.4, 11.5, 107.2, 109.9, 118.7, 119.5, 126.6, 128.0, 131.4, 135.4. HRMS: calcd for $C_{10}H_9ClN$: 178.0428 $[M - H]^-$, found: 178.0426.

6-Fluoro-2,3-dimethylindole. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.15 (s, 3H), 2.23 (s, 3H), 6.78–6.84 (m, 2H), 7.30 (dd, 1H, J = 9.2, 5.3 Hz), 7.55 (s, NH). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 8.3, 11.3, 96.5 (d, J = 26 Hz), 106.8, 107.1 (d, J = 24 Hz), 118.3 (d,

J = 10 Hz), 125.9, 130.9 (d, J = 3.6 Hz), 134.9 (d, J = 12 Hz), 159.2 (d, J = 235 Hz). HRMS: calcd for $C_{10}H_9FN$: 162.0724 [M − H]$^-$, found: 162.0722.

2,3,7-Trimethylindole. mp 64–65 °C (lit.[19] 79 °C, lit.[24,30] 75–76 °C). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.21 (s, 3H), 2.37 (s, 3H), 2.44 (s, 3H), 6.89–6.93 (m, 1H), 7.00 (dd, 1H, J = 7.8, 6.3 Hz), 7.32 (d, 1H, J = 7.8 Hz), 7.60 (br s, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 8.6, 11.6, 16.5, 107.6, 115.7, 119.1, 119.2, 121.5, 128.8, 130.2, 134.5. MS: m/z 159 [M]. NMR data are in accordance with literature values.[24]

7-Methoxy-2,3-dimethylindole. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.20 (s, 3H), 2.33 (s, 3H), 3.93 (s, 3H), 6.58 (d, 1H, J = 7.5 Hz), 6.99 (t, 1H, J = 7.8 Hz), 7.90 (d, 1H, J = 7.8 Hz), 7.90 (br s, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 8.7, 11.5, 55.3, 101.2, 107.5, 111.0, 119.3, 125.2, 130.2, 130.7, 145.3. HRMS: calcd for $C_{11}H_{12}NO$: 174.0924 [M − H]$^-$, found: 174.0928.

2,3-Dimethyl-1*H*-benzo[*g*]indole. mp 149 °C (lit.[19] 153–155 °C). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.29 (s, 3H), 2.45 (s, 3H), 7.36 (ddd, 1H, J = 7.8, 6.9, 1.2 Hz), 7.45 (ddd, 1H, J = 8.2, 6.9, 1.2 Hz), 7.47 (d, 1H, J = 8.2 Hz), 7.61 (d, 1H, J = 8.4 Hz), 7.87–7.95 (m, 2H), 8.40 (br s, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 8.6, 11.6, 118.6, 119.0, 119.7, 121.2, 123.0, 125.1, 128.8, 129.9. MS: m/z 195 [M]. ^{1}H NMR data are in accordance with literature values.[31]

1,2-Dimethyl-3*H*-benzo[*e*]indole. mp 118–121 °C (lit.[32] 131 °C). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.39 (s, 3H), 2.61 (s, 3H), 7.37 (d, 1H, J = 8.7 Hz), 7.37 (ddd, 1H, J = 8.4, 6.9, 1.5 Hz), 7.50 (d, 1H, J = 8.7 Hz), 7.51 (ddd, 1H, J = 8.4, 6.9, 1.5 Hz), 7.90 (d, 1H, J = 8.8 Hz), 7.94 (br s, 1H), 8.49 (d, 1H, J = 8.4 Hz). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 11.5, 12.4, 109.7 (2C), 112.2, 121.8, 122.5, 131.1, 125.2, 128.6, 128.9, 129.1, 129.6, 131.3. MS: m/z 195 [M].

2-Ethyl-3-methylindole (major)[a] and 3-ethyl-2-methylindole (minor)[b]. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.21 (t, 3H, J = 7.8 Hz)[b], 1.26 (t, 3H, J = 7.8 Hz)[a], 2.23 (s, 3H)[a], 2.35 (s, 3H)[b], 2.70 (q, 2H, J = 7.8 Hz)[b], 2.74 (q, 2H, J = 7.5 Hz)[a], 7.04–7.14 (m, 4H)[a,b], 7.22–7.28 (m, 2H)[a,b], 7.46–7.54 (m, 2H)[a,b], 7.68 (br s, 2H)[a,b]. ^{13}C NMR ($CDCl_3$, 75 MHz): δ 8.3[a], 11.5[b], 14.0[a], 15.4[b], 17.3[b], 19.4[a], 106.2[a], 110.1[a,b], 113.9[b], 118.0[a,b], 119.0[a,b], 120.9[a,b], 128.5[b], 129.4[a], 130.1[b], 135.0[a,b], 136.4[a]. MS: m/z: 159 [M]. NMR data for 2-ethyl-3-methylindole[a] are in accordance with literature values.[33] NMR data for 3-ethyl-2-methylindole[b] are in accordance with literature values.[24]

2-Butyl-3-methylindole. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.92 (t, 3H, J = 7.3 Hz), 1.35 (sextet, 2H, J = 7.3 Hz), 1.57 (p, 2H, J = 7.2 Hz), 2.22 (s, 3H), 2.66 (t, 3H, J = 7.3 Hz), 7.09 (m, 2H), 7.20 (d, 1H, J = 7.1 Hz), 7.50 (d, 1H, J = 7.5 Hz), 7.55 (br s, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 8.4, 13.9, 22.4, 25.8, 31.8, 106.7, 110.1, 118.0, 118.9, 120.8, 129.3, 135.0, 135.3. MS: m/z 187 [M]. NMR data are in accordance with literature values.[34]

2-Isopropyl-3-methylindole. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.31 (d, 6H, J = 7.0 Hz), 2.25 (s, 3H), 3.25 (septet, 1H, J = 7.0 Hz), 7.04–7.14 (m, 2H), 7.26–7.30 (m, 1H), 7.47–7.50 (m, 1H), 7.72 (br s, NH). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 8.4, 22.3, 25.6, 105.2, 110.2, 118.0, 119.0, 120.8, 129.4, 134.8, 140.2. MS: m/z 173 [M]. NMR data are in accordance with literature values.[35]

3-Methyl-2-phenylindole. mp 87–90 °C (lit.[22] 90–91 °C). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.46 (s, 3H), 7.11–7.24 (m, 2H), 7.32–7.36 (m, 2H), 7.44–7.50 (m, 2H), 7.55–7.62 (m, 3H), 8.00 (br s, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 9.6, 108.6, 110.6, 119.0, 119.5, 122.2, 127.2, 127.7, 128.7, 129.9, 133.2, 134.0, 135.7. MS: m/z 207 [M]. NMR data are in accordance with literature values.[20a,23]

1,2,3,4-Tetrahydrocarbazole. mp 111–113 °C (lit.[19] 114 °C, lit.[30] 116–118 °C). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.80–1.94 (m, 4H), 2.64–2.74 (m, 4H), 7.02–7.14 (m, 2H), 7.20–7.26 (m, 1H), 7.42–7.48 (m, 1H), 7.52–7.66 (br s, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 21.4, 23.7, 23.8, 110.6, 110.8, 118.2, 119.5, 121.4, 128.3, 134.5, 136.1. MS: m/z 171 [M]. NMR data are in accordance with literature values.[23,24]

2,3-Diphenylindole. mp 108–109 °C (lit.[36] 109 °C). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.10–7.50 (m, 13H), 7.69 (d, 1H, J = 7.8 Hz), 8.18 (br s, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 110.9, 115.0, 119.7, 120.4, 122.7, 126.2, 127.7, 128.1, 128.5, 128.7 (2C), 130.1, 132.6, 134.0, 135.0, 135.8 ppm. MS: m/z 269 [M]. NMR data are in accordance with literature values.[36]

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